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(54) Title: PEPTIDE NUCLEIC ACIDS

(57) Abstract

A novel class of compounds, known as peptide nucleic acids, bind complementary ssDNA and RNA strands more strongly than a corresponding DNA. The peptide nucleic acids generally comprise ligands such as naturally occurring DNA bases attached to a peptide backbone through a suitable linker.

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PEPTIDE NUCLEIC ACIDS

RELATED APPLICATIONS

This application is a continuation-in-part of the following Danish Patent Applications: No. 986/91, filed May 24, 1991, No. 987/91, filed May 24, 1991, and No. 510/92, filed April 15, 1992. The entire disclosure of each application is incorporated herein by reference.

FIELD OF THE INVENTION

This invention is directed to compounds that are not polynucleotides yet which bind to complementary DNA and RNA strands more strongly the corresponding DNA. In particular, the invention concerns compounds wherein naturally-occurring nucleobases or other nucleobase-binding moieties are covalently bound to a polyamide backbone.

BACKGROUND OF THE INVENTION

Oligodeoxyribonucleotides as long as 100 base pairs (bp) are routinely synthesized by solid phase methods using commercially available, fully automatic synthesis machines. The chemical synthesis of oligoribonucleotides, however, is far less routine. Oligoribonucleotides also are much less stable than oligodeoxyribonucleotides, a fact which has contributed to the more prevalent use of oligodeoxyribonucleotides in medical and biological research directed to, for exampl , gene therapy or the regulation of transcription r translation.

The function of a gene starts by transcription of its information to a messenger RNA (mRNA) which, by interaction with the ribosomal complex, directs the synthesis of a protein coded for by its sequence. The synthetic process is known as translation. Translation requires the presence of various co-factors and building blocks, the amino acids, and their transfer RNAs (tRNA), all of which are present in normal cells.

Transcription initiation requires specific recognition of a promoter DNA sequence by the RNA-synthesizing enzyme, RNA polymerase. In many cases in prokaryotic cells, and probably in all cases in eukaryotic cells, this recognition is preceded by sequence-specific binding of a protein transcription factor to the promoter. Other proteins which bind to the promoter, but whose binding prohibits action of RNA polymerase, are known as repressors. Thus, gene activation typically is regulated positively by transcription factors and negatively by repressors.

Most conventional drugs function by interaction with and modulation of one or more targeted endogenous proteins, *e.g.*, enzymes. Such drugs, however, typically are not specific for targeted proteins but interact with other proteins as well. Thus, a relatively large dose of drug must be used to effectively modulate a targeted protein. Typical daily doses of drugs are from 10^{-5}-10^{-1} millimoles per kilogram of body weight or 10^{-3}-10 millimoles for a 100 kilogram person. If this modulation instead could be effected by interaction with and inactivation of mRNA, a dramatic reduction in the necessary amount of drug necessary could likely be achieved, along with a corresponding reduction in side effects. Further reductions could be effected if such interaction could be rendered site- specific. Given that a functioning gene continually produces mRNA, it would thus be even more advantageous if gene transcription could be arrest d in its entirety.

Oligodeoxynucleotides ffer such opportunities. For example, synthetic ligode xynucle tides could be used as

antisense pr b s to bl ck and eventually lead to the breakdown of mRNA. Thus, synthetic DNA c uld suppress translation *in vivo*. It als may be possible to modulate the genome of an animal by, for example, triple helix formation using oligonucleotides or other DNA recognizing agents. However, there are a number of drawbacks associated with triple helix formation. For example, it can only be used for homopurine sequences and it requires unphysiologically high ionic strength and low pH.

Furthermore, unmodified oligonucleotides are unpractical both in the antisense approach and in the triple helix approach because they have short *in vivo* half-lives, they are difficult to prepare in more than milligram quantities and, thus, are prohibitively costly, and they are poor cell membrane penetrators.

These problems have resulted in an extensive search for improvements and alternatives. For example, the problems arising in connection with double-stranded DNA (dsDNA) recognition through triple helix formation have been diminished by a clever "switch back" chemical linking whereby a sequence of polypurine on one strand is recognized, and by "switching back", a homopurine sequence on the other strand can be recognized. *See, e.g.*, McCurdy, Moulds, and Froehler, *Nucleosides*, in press. Also, good helix formation has been obtained by using artificial bases, thereby improving binding conditions with regard to ionic strength and pH.

In order to improve half life as well as membrane penetration, a large number of variations in polynucleotide backbones has been undertaken, although so far not with desired results. These variations include the use of methylphosphonates, monothiophosphates, dithiophosphates, phosphoramidates, phosphate esters, bridged phosphoroamidates, bridged phosphorothioates, bridged methylenephosphonates, dephospho internucleotide analogs with siloxane bridges, carbonate bridges, carboxymethyl st r bridges, acetamide bridges, carbamate bridges, thioether, sulf xy, sulf no

bridges, various "plastic" DNAs, α-anomeric bridges, and borane derivatives.

International patent application WO 86/05518 broadly claims a polymeric composition effective to bind to a single-stranded polynucleotide containing a target sequence of bases. The composition is said to comprise non-homopolymeric, substantially stereoregular polymer molecules of the form:

$$\begin{array}{ccccccc} R_1 & & R_2 & & R_3 & & R_n \\ B & - & B & - & B & - \ldots & B, \end{array}$$

where:

(a) R_1-R_n are recognition moieties selected from purine, purine-like, pyrimidine, and pyrimidine like heterocycles effective to bind by Watson/Crick pairing to corresponding, in-sequence bases in the target sequence;

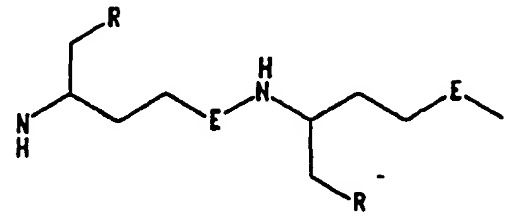
(b) n is such that the total number of Watson/Crick hydrogen bonds formed between a polymer molecule and target sequence is at least about 15;

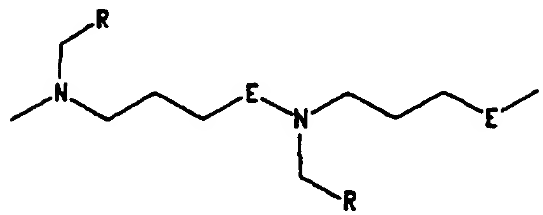
(c) B - B are backbone moieties joined predominantly by chemically stable, substantially uncharged, predominantly achiral linkages;

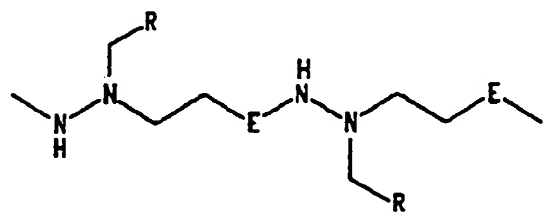
(d) the backbone moiety length ranges from 5 to 7 atoms if the backbone moieties have a cyclic structure, and ranges from 4 to 6 atoms if the backbone moieties have an acyclic structure; and

(e) the backbone moieties support the recognition moieties at position which allow Watson/Crick base pairing between the recognition moieties and the corresponding, in-sequence bases of the target sequence.

According to WO 86/05518, the recognition moieties are various natural nucleobases and nucleobase-analogs and the backbone moieties are either cyclic backbone moieties comprising furan or morpholine rings r acyclic backb ne moieties of the following forms:







where E is -CO- or $-SO_2-$. The specification of the application provides general descriptions for the synthesis of subunits, for backbone coupling reactions, and for polymer assembly strategies. However, the specification provides no example wherein a claimed compound or structure is actually prepared. Although WO 86/05518 indicates that the claimed polymer compositions can bind target sequences and, as a result, have possible diagnostic and therapeutic applications, the application contains no data relating to the binding affinity of a claimed polymer.

International patent application WO 86/05519 claims diagnostic reagents and systems that comprise polymers described in WO 86/05518, but attached to a solid support. WO 86/05519 also provides no examples concerning actually preparation of a claimed diagnostic reagent, much less data showing the diagnostic efficiency of such a reagent.

Int rnational patent application WO 89/12060 claims various building blocks for synthesizing lig nucleotide analogs, as well as oligonucl otide anal gs formed by joining

such building blocks in a defined sequence. The building blocks may be either "rigid" (containing a ring) or "flexible" (lacking a ring). In both cases the building blocks contain a hydroxy group and a mercapto group, through which the building blocks are said to join to form oligonucleotide analogs. The linking moiety in the oligonucleotide analogs is selected from the group consisting of sulfide (-S-), sulfoxide (-SO-), and sulfone ($-SO_2-$). WO 89/12060 provides a general description concerning synthesis of the building blocks and coupling reactions for the synthesis of oligonucleotide analogs, along with experimental examples describing the preparation of building blocks. However, the application provides no examples directed to the preparation of a claimed oligonucleotide analog and no data confirming the specific binding of an oligonucleotide analog to a target oligonucleotide.

Furthermore, oligonucleotides or their derivatives have been linked to intercalators in order to improve binding, to polylysine or other basic groups in order to improve binding both to double-stranded and single-stranded DNA, and to peptides in order to improve membrane penetration. However, such linking has not resulted in satisfactory binding for either double-stranded or single-stranded DNA. Other problems which resulted from, for example, methylphosphonates and monothiophosphates were the occurrence of chirality, insufficient synthetic yield or difficulties in performing solid phase assisted syntheses.

In most cases only a few of these modifications could be used. Even then, only short sequences -- often only dimers -- or monomers could be generated. Furthermore, the oligomers actually produced have rarely been shown to bind to DNA or RNA or have not been examined biologically.

The great majority of these backbone modifications led to decreased stability for hybrids formed between the modified oligonucleotide and its complementary native oligonucleotide, as assay d by measuring T_m values. Consequently, it is generally understood in the art that backb ne m difications

destabilize such hybrids, *i.e.*, result in lower T_m values, and should be kept to a minimum.

OBJECTS OF THE INVENTION

It is one object of the present invention to provide compounds that bind ssDNA and RNA strands to form stable hybrids therewith.

It is a further object of the invention to provide compounds that bind ssDNA and RNA strands more strongly the corresponding DNA.

It is another object to provide compounds wherein naturally-occurring nucleobases or other nucleobase-binding moieties are covalently bound to a peptide backbone.

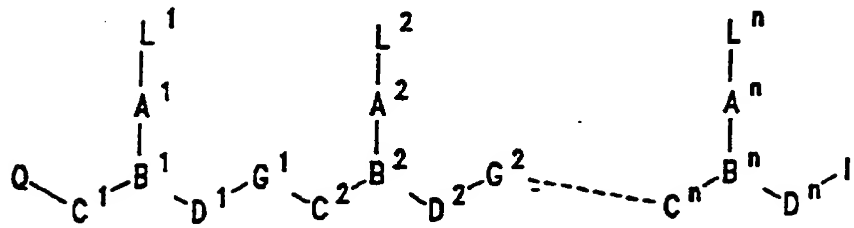
It is yet another object to provide compounds other than RNA that can bind one strand of a double-stranded polynucleotide, thereby displacing the other strand.

It is still another object to provide therapeutic and prophylactic methods that employ such compounds.

SUMMARY OF THE INVENTION

The present invention provides a novel class of compounds, known as peptide nucleic acids (PNAs), that bind complementary ssDNA and RNA strands more strongly than a corresponding DNA. The compounds of the invention generally comprise ligands linked to a peptide backbone via an aza nitrogen. Representative ligands include either the four main naturally occurring DNA bases (*i.e.*, thymine, cytosine, adenine or guanine) or other naturally occurring nucleobases (*e.g.*, inosine, uracil, 5-methylcytosine or thiouracil) or artificial bases (*e.g.*, bromothymine, azaadenines or azaguanines, *etc.*) attached to a peptide backbone through a suitable linker.

In certain preferred embodiments, the peptide nucleic acids of the invention have the general formula (I):



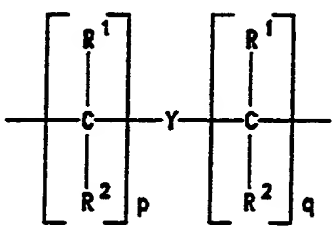
(I)

wherein:

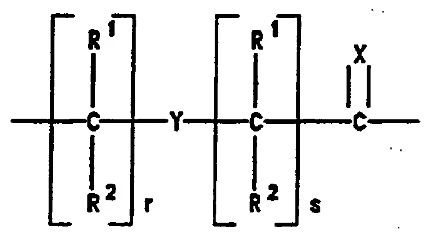
n is at least 2,

each of L^1-L^n is independently selected from the group consisting of hydrogen, hydroxy, $(C_1$-$C_4)$alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, and reporter ligands, at least one of L^1-L^n being a naturally occurring nucleobase, a non-naturally occurring nucleobase, a DNA intercalator, or a nucleobase-binding group;

each of A^1-A^n is a single bond, a methylene group or a group of formula (IIa) or (IIb):



or



(IIa) (IIb)

where:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR^4;

each of p and q is zero or an integer from 1 to 5, the sum p+q being not more than 10;

each of r and s is zero or an integer from 1 to 5, the sum r+s being not more than 10;

ach R^1 and R^2 is independently selected from the group consisting of hydrogen, $(C_1$-$C_4)$alkyl which may be hydroxy- or alkoxy- or alkylthio-substituted, hydr xy, alk xy, alkylthio, amino and halogen; and

ach R^3 and R^4 is independently selected from the group c nsisting of hydrogen, (C_1-C_4)alkyl, hydr xy- r alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio and amino;

each of B^1-B^n is N or R^3N^+, where R^3 is as defined above;

each of C^1-C^n is CR^6R^7, CHR^6CHR^7 or $CR^6R^7CH_2$, where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_2-C_6)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6)alkoxy, (C_1-C_6)alkylthio, NR^3R^4 and SR^5, where R^3 and R^4 are as defined above, and R^5 is hydrogen, (C_1-C_6)alkyl, hydroxy-, alkoxy-, or alkylthio- substituted (C_1-C_6)alkyl, or R^6 and R^7 taken together complete an alicyclic or heterocyclic system;

each of D^1-D^n is CR^6R^7, $CH_2CR^6R^7$ or CHR^6CHR^7, where R^6 and R^7 are as defined above;

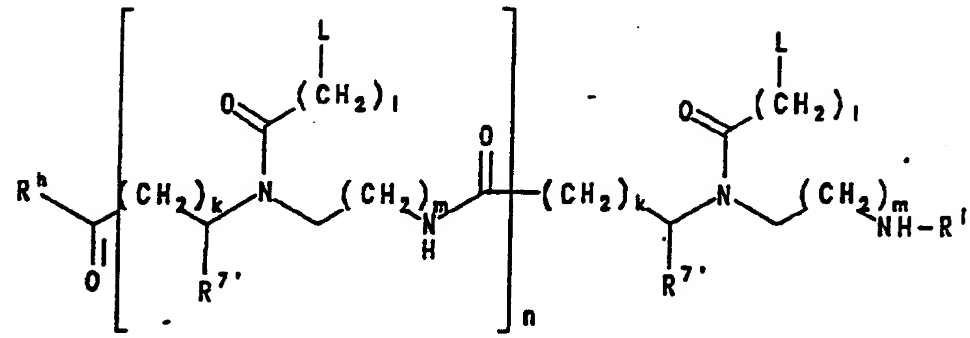
each of G^1-G^{n-1} is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO_2-$,Y in either orientation, where R^3 is as defined above;

Q is $-CO_2H$, -CONR'R'', $-SO_3H$ or $-SO_2NR'R''$ or an activated derivative of $-CO_2H$ or $-SO_3H$; and

I is -NHR'''R'''' or -NR'''C(O)R'''', where R', R", R''' and R'''' are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, oligonucleotid s and soluble and non-soluble polymers.

The peptide nucleic acids of the invention differ fr m those disclosed in WO 86/05518 in that their recognition moieties are attached to an aza nitrogen atom in the backbone, rather than to an amide nitrogen atom, a hydrazine moiety r a carbon atom in the backbone.

Preferred peptid nucleic acids have general formula (III):



(III)

wherein:

each L is independently selected from the group consisting of hydrogen, phenyl, heterocyclic moieties, naturally occurring nucleobases, and non-naturally occurring nucleobases;

each $R^{7'}$ is independently selected from the group consisting of hydrogen and the side chains of naturally occurring alpha amino acids;

n is an integer from 1 to 60;

each of k, l and m is independently zero or an integer from 1 to 5;

R^h is OH, NH_2 or $-NHLysNH_2$; and

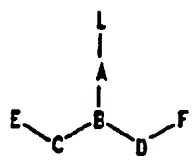
R^i is H or $COCH_3$.

Particularly preferred are compounds having formula (III) wherein each L is independently selected from the group consisting of the nucleobases thymine (T), adenine (A), cytosine (C), guanine (G) and uracil (U), k and m are zero or 1, and n is an integer from 1 to 30, in particular from 4 to 20. An example of such a compound is provided in Figure 1, which shows the structural similarity between such compounds and single-stranded DNA.

The peptide nucleic acids of the invention are synthesized by adaptation f standard peptide synthesis pr cedures, either in soluti n or on a s lid phase. The synthons used are specially designed monomer amino acids or their activated derivatives, pr tected by standard protecting

groups. The ligonucl otid analogs also can be synthesized by using the corresponding diacids and diamines.

Thus, the novel monomer synthons according to th invention are selected from the group consisting of amino acids, diacids and diamines having general formulae: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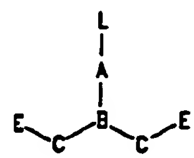
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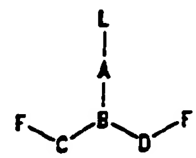
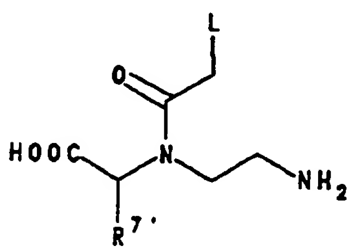


(IV) (V) (VI)

wherein L, A, B, C and D are as defined above, except that any amino groups therein may be protected by amino protecting groups; E is COOH, CSOH, SOOH, SO_2OH or an activated derivative thereof; and F is NHR^3 or $NPgR^3$, where R^3 is as defined above and Pg is an amino protecting group.

Preferred monomer synthons according to the invention are amino acids having formula (VII):



(VII)

or amino-protected and/or acid terminal activated derivatives thereof, wherein L is selected from the group consisting f hydrogen, phenyl, heterocyclic moieties, naturally occurring nucleobases, non-naturally occurring nucleobases, and protected derivatives thereof; and $R^{7'}$ is independently selected from the group consisting of hydrogen and the side chains of naturally occurring alpha amino acids. Especially preferred are such synthons having formula (VII) wherein $R^{7'}$ is hydrogen and L is selected from the group consisting f the nucle bases thymine (T), adenine (A), cytosine (C), guanine (G) and uracil (U) and protected derivatives thereof.

Unexpectedly, these comp unds also are able to recognize duplex DNA by displacing on strand, thereby presumably generating a double helix with the other one. Such recognition can take place to dsDNA sequences 5-60 base pairs long. Sequences between 10 and 20 bases are of interest since this is the range within which unique DNA sequences of prokaryotes and eukaryotes are found. Reagents which recognize 17-18 bases are of particular interest since this is the length of unique sequences in the human genome. The compounds of the invention also should be able to form triple helices with dsDNA.

Whereas the improved binding of the compounds of the invention should render them efficient as antisense agents, it is expected that an extended range of related reagents may cause strand displacement, now that this surprising and unexpected new behavior of dsDNA has been discovered.

Thus, in one aspect, the present invention provides methods for inhibiting the expression of particular genes in the cells of an organism, comprising administering to said organism a reagent as defined above which binds specifically to sequences of said genes.

Further, the invention provides methods for inhibiting transcription and/or replication of particular genes or for inducing degradation of particular regions of double stranded DNA in cells of an organism by administering to said organism a reagent as defined above.

Still further, the invention provides methods for killing cells or virus by contacting said cells or virus with a reagent as defined above which binds specifically to sequences of the genome of said cells or virus.

BRIEF DESCRIPTION F THE DRAWINGS

The numerous objects and advantages of the present inventi n may be better underst od by those skilled in the art by reference to the accompanying figures, in which:

Figure 1 shows a naturally occurring deoxyribooligonucleotide (A) and a peptide nucleic acid (PNA) of the invention (B).

Figure 2 provides examples of naturally occurring and non-naturally occurring nucleobases for DNA recognition and reporter groups.

Figure 3 provides a schematic illustration of (a) photocleavage by Acr^1-$(Taeg)_{10}$-Lys-NH_2 (Acr-T10-LysNH_2); (b) photofootprint by the diazo-linked acridine of Acr^1-$(Taeg)_{10}$-Lys-NH_2 and preferred $KMnO_4$-cleavage; and (c) S_1-nuclease enhanced cleavage and (d) micrococcus nuclease cleavage f Acr^1-$(Taeg)_{10}$-Lys-NH_2 binding site.

Figure 4 provides examples of PNA monomer synthons of the invention.

Figure 5 shows the Acr^1 ligand and a PNA, Acr^1-$(Taeg)_{10}$-Lys-NH_2.

Figure 6 provides a general scheme for the preparation of monomer synthons.

Figure 7 provides a general scheme for the preparation of the Acr^1 ligand.

Figure 8 provides a general scheme for solid-phase PNA synthesis illustrating the preparation of linear unprotected PNA amides.

Figure 9 shows analytical HPLC chromatograms of: (A) crude H-$[Taeg]_{15}$-NH_2 after HF-cleavage (before lyophilization); (B) crude Acr^1-$[Taeg]_{15}$-NH_2 after HF-cleavage (before lyophilization); and (C) purified Acr^1-$[Taeg]_{15}$-NH_2. Buffer A, 5% CH_3CN/95% H_2O/0.0445% TFA; buffer B, 60% CH_3CN/40% H_2O/0.0390% TFA; linear gradient, 0-100% of B in 30 min; flow rate, 1.2 ml/min; column, Vydac C_{18} (5 μm, 0.46 x 25 cm).

Figure 10 shows analytical HPLC chromat grams of: (A) purified H-$[Taeg]_{10}$-Lys-NH_2 and (B) purified H-$[Taeg]_5$-Caeg-$[Taeg]_4$-Lys-NH_2 empl ying the same conditi ns as in Figure 9.

Figures 11a and 11b show binding of AcrT10-Lys to dA_{10}. 5'-^{32}P-labeled oligonucleotide (1) (5'-GATCCA_{10}G) was incubated in the absence or presence of Acr-T10-LysNH_2 and in the absence or presence of oligonucleotide (2) (5'-GATCCT_{10}G) and the samples were analyzed by polyacrylamide gel electrophoresis (PAGE) and autoradiography under "native conditions" (Figure 11a) or under "denaturing conditions" (Figure 11b).

Figures 12a-c show chemical, photochemical and enzymatic probing of dsDNA-Acr-T10-LysNH_2 complex. Complexes between Acr-T10-LysNH_2 and a ^{32}P-endlabeled DNA fragment containing a dA_{10}/dT_{10} target sequence were probed by affinity photocleavage (Figure 12a, lanes 1-3; Figure 12b, lanes 1-3), photofootprinting (Figure 12a, lanes 5-6), potassium permanganate probing (Figure 12b, lanes 4-6) or probing by staphylococcus nuclease (Figure 12b, lanes 8-10) or by nuclease S_1 (Figure 12c). Either the A-strand (Figure 12a) or the T-strand (Figures 12b,c) was probed.

Figure 13 provides a procedure for the synthesis of protected PNA synthons.

Figure 14 provides a procedure for the synthesis of a protected adenine monomer synthon.

Figure 15 provides a procedure for the synthesis of a protected guanine monomer synthon.

Figure 16 provides examples of PNA backbone alterations.

Figure 17 provides a procedure for synthesis of thymine monomer synthons with side chains corresponding to the normal amino acids.

Figures 18a and 18b provide procedures for synthesis of an aminopropyl analogue and a propionyl analogue, respectively, of a thymine monomer synthon.

Figure 19 provides a procedure for synthesis of an aminoethyl-β-alanine analogue of thymine monomer synthon.

Figur 20 shows a PAGE autoradiograph demonstrating that PNAs-T_{10}, -T_9C and -T_8C_2 bind to double stranded DNA with high sequenc specificity.

Figure 21 sh ws a graph based on densit metric scanning f PAGE aut radi graphs demonstrating the kinetics of the binding f PNA-T_{10} to a double stranded target.

Figure 22 shows a graph based on densitometric scanning of PAGE autoradiographs demonstrating the thermal stabilities of PNAs of varying lengths bound to an A_{10}/T_{10} double stranded DNA target.

Figure 23 shows an electrophoretic gel staining demonstrating that restriction enzyme activity towards DNA is inhibited when PNA is bound proximal to the restriction enzyme recognition site.

Figure 24 shows a PAGE autoradiograph demonstrating that ^{125}I-labeled PNA-T_{10} binds to a complementary dA_{10} oligonucleotide.

Figure 25 shows a peptide nucleic acid according to the invention.

Figure 26 shows the direction of synthesis for a peptide nucleic acid according to the invention.

Figure 27 provides a test for the tosyl group as a nitrogen protecting group in the synthesis of peptide nucleic acids.

DETAILED DESCRIPTION OF THE INVENTION

In the oligonucleotide analogs and monomer synthons according to the invention, ligand L is primarily a naturally occurring nucleobase attached at the position found in nature, i.e., position 9 for adenine or guanine, and position 1 for thymine or cytosine. Alternatively, L may be a non-naturally occurring nucleobase (nucleobase analog), another base-binding moiety, an aromatic moiety, (C_1-C_4)alkanoyl, hydroxy or even hydrogen. Some typical nucleobase ligands and illustrative synthetic ligands are shown in Figure 2. Furthermore, L can be a DNA intercalator, a reporter ligand such as, for example, a fluorophor, radio label, spin label, hapten, or a protein-recognizing ligand such as biotin.

In m n mer synthons, L may be blocked with protecting gr ups. This is illustrated in Figure 4, where Pg^1 is an

acid, a bas or a hydrogenolytically or ph tochemically cleavable prot cting gr up such as, for example, t-butoxycarbonyl (Boc), fluorenylmethyloxycarbonyl (Fmoc) or 2-nitrobenzyl (2Nb).

Linker A can be a wide variety of groups such as $-CR^1R^2CO-$, $-CR^1R^2CS-$, $-CR^1R^2CSe-$, $-CR^1R^2CNHR^3-$, $-CR^1R^2C{=}CH_2-$ and $-CR^1R^2C{=}C(CH_3)_2-$, where R^1, R^2 and R^3 are as defined above. Preferably, A is methylenecarbonyl ($-CH_2CO-$). Also, A can be a longer chain moiety such as propanoyl, butanoyl or pentanoyl, or corresponding derivative, wherein O is replaced by another value of X or the chain is substituted with R^1R^2 or is heterogenous, containing Y. Further, A can be a (C_2-C_6)alkylene chain, a (C_2-C_6)alkylene chain substituted with R^1R^2 or can be heterogenous, containing Y. In certain cases, A can just be a single bond.

In the preferred form of the invention, B is a nitrogen atom, thereby presenting the possibility of an achiral backbone. B can also be R^3N^+, where R^3 is as defined above.

In the preferred form of the invention, C is $-CR^6R^7-$, but can also be a two carbon unit, *i.e.* $-CHR^6CHR^7-$ or $-CR^6R^7CH_2-$, where R^6 and R^7 are as defined above. R^6 and R^7 also can be a heteroaryl group such as, for example, pyrrolyl, furyl, thienyl, imidazolyl, pyridyl, pyrimidinyl, indolyl, or can be taken together to complete an alicyclic system such as, for example, 1,2-cyclobutanediyl, 1,2-cyclopentanediyl or 1,2-cyclohexanediyl.

In the preferred form of the invention, E in the monomer synthon is COOH or an activated derivative thereof, and G in the oligomer is $-CONR^3-$. As defined above, E may also be CSOH, SOOH, SO_2OH or an activated derivative thereof, whereby G in the oligomer becomes $-CSNR^3-$, $-SONR^3-$and $-SO_2NR^3-$, respectively. The activation may, for example, be achieved using an acid anhydride or an active ester derivative, wherein hydr gen in the groups represented by E is replaced by a leaving group suited f r generating the growing backbone.

The amin acids which form the backbone may be identical or different. We have found that those based n 2-

aminoethylglycine are especially well suited to th purpose f the invention.

In s me cas s it may be of interest to attach ligands at either terminus (Q, I) to modulate the binding characteristics of the PNAs. Representative ligands include DNA intercalators which will improve dsDNA binding or basic groups, such as lysine or polylysine, which will strengthen the binding of PNA due to electrostatic interaction. To decrease negatively charged groups such as carboxy and sulfo groups could be used. The design of the synthons further allows such other moieties to be located on non-terminal positions.

In a further aspect of the invention, the PNA oligomers are conjugated to low molecular effector ligands such as ligands having nuclease activity or alkylating activity or reporter ligands (fluorescent, spin labels, radioactive, protein recognition ligands, for example, biotin or haptens). In a further aspect of the invention, the PNAs are conjugated to peptides or proteins, where the peptides have signaling activity and the proteins are, for example, enzymes, transcription factors or antibodies. Also, the PNAs can be attached to water-soluble or water-insoluble polymers. In another aspect of the invention, the PNAs are conjugated to oligonucleotides or carbohydrates. When warranted, a PNA oligomer can be synthesized onto some moiety (*e.g.*, a peptide chain, reporter, intercalator or other type of ligand-containing group) attached to a solid support.

Such conjugates can be used for gene modulation (*e.g.*, gene targeted drugs), for diagnostics, for biotechnology, and for scientific purposes.

As a further aspect of the invention, PNAs can be used to target RNA and ssDNA to produce both antisense-type gene regulating moieties and hybridization probes for the identification and purification of nucleic acids. Furthermore, the PNAs can be modified in such a way that they can form triple helices with dsDNA. Reagents that bind sequence-specifically t dsDNA have applications as g ne

targeted drugs. Thes are foreseen as extremely useful drugs for tr ating diseas s like cancer, AIDS and ther virus infections, and may also prove effective for treatment of some genetic diseases. Furthermore, these reagents may be used for research and in diagnostics for detection and isolation of specific nucleic acids.

The triple helix principle is believed to be the only known principle in the art for sequence-specific recognition of dsDNA. However, triple helix formation is largely limited to recognition of homopurine-homopyrimidine sequences. Strand displacement is superior to triple helix recognition in that it allows for recognition of any sequence by use of the four natural bases. Also, in strand displacement recognition readily occurs at physiological conditions, that is, neutral pH, ambient (20-40 C) temperature and medium (100-150 mM) ionic strength.

Gene targeted drugs are designed with a nucleobase sequence (containing 10-20 units) complementary to the regulatory region (the promoter) of the target gene. Therefore, upon administration of the drug, it binds to the promoter and block access thereto by RNA polymerase. Consequently, no mRNA, and thus no gene product (protein), is produced. If the target is within a vital gene for a virus, no viable virus particles will be produced. Alternatively, the target could be downstream from the promoter, causing the RNA polymerase to terminate at this position, thus forming a truncated mRNA/protein which is nonfunctional.

Sequence-specific recognition of ssDNA by base complementary hybridization can likewise be exploited to target specific genes and viruses. In this case, the target sequence is contained in the mRNA such that binding of the drug to the target hinders the action of ribosomes and, consequently, translation of the mRNA into protein. The peptide nucleic acids of the inv ntion are superi r t pri r agents in that th y have significantly higher affinity for complementary ssDNA. Also, they possess n charge and water soluble, which should facilitate cellular uptake, and th y contain amides of

n n-biol gical amino acids, which sh uld make them biostable and resistant to enzymatic d gradation by, f r example, proteases.

Certain biochemical/biological properties of PNA oligomers are illustrated by the following experiments.

1. Sequence discrimination at the dsDNA level (Example 63, Figure 20).

Using the S_1-nuclease probing technique, the discrimination of binding of the T_{10}, T_5CT_4 (T_9C) & $T_2CT_2CT_4$ (T_8C_2) PNA to the recognition sequences A_{10}, A_5GA_4 (A_9G) & $A_2GA_2GA_4$ (A_8G_2) cloned into the *Bam*HI, *Sal*I or *Pst*I site of the plasmid pUC19 was analyzed. The results (Figure 20) show that the three PNAs bind to their respective recognition sequences with the following relative efficiencies: PNA - T10: $A_{10} > A_9G >> A_8G_2$, PNA -T_9C: $A_9G > A_{10} \sim A_8G_2$, PNA - T_8C_2: $A_8G_2 \geq A_9G >> A_{10}$. Thus at 37°C one mismatch out of ten gives reduced efficiency (5-10 times estimated) whereas two mismatches are not accepted.

2. Kinetics of PNA-T_{10} - dsDNA strand displacement complex formation (Example 66, Figure 21).

Complex formation was probed by S_1-nuclease at various times following mixing of PNA and ^{32}P-endlabeled dsDNA fragment (Figure 21).

3. Stability of PNA-dsDNA complex (Example 67, Figure 22)

Complexes between PNA-T_n and ^{32}P-dsDNA (A_{10}/T_{10}) target were formed (60 min, 37°C). The complexes were then incubated at the desired temperature in the presence of excess oligo-dA_{10} for 10 min, cooled to RT and probed with $KMnO_4$. Th results (Figure 22) show that the thermal stability of the PNA-dsDNA complexes mirror that of the PNA oligonucleotide complexes in terms of "Tm".

4. Inhibition of restriction enzyme cleavage by PNA (Example 65, Figure 23)

The plasmid construct, pT10, contains a dA_{10}/dT_{10} tract cloned into the *Bam*HI site in pUC19. Thus, cleavage of pT10 with *Bam*HI and *Pvu*II results in two small DNA fragments of 211 and 111 bp, respectively. In the presence of PNA-T_{10}, a 336 bp fragment is obtained corresponding t cleavage nly by

PvuII (Figure 23). Thus cleavage by *BamHI* is inhibited by PNA bound proximal to the r striction enzyme site. Th results also show that the PNA-dsDNA complex can be formed in 100% yield. Similar results were obtained using the pT8C2 plasmid and PNA-T8C2.

5. Binding of ^{125}I-labeled PNA to oligonucleotides (Example 63, Figure 24)

A Tyr-PNA-T_{10}-Lys-NH_2 was labeled with ^{125}I using Na^{125}I and chloramine-T and purified by HPLC. The ^{125}I-PNA-T_{10} was shown to bind to oligo-dA_{10} by PAGE and autoradiography (Figure 24). The binding could be competed by excess denatured calf thymus DNA.

The sequence-specific recognition of dsDNA is illustrated by the binding of a PNA, consisting of 10 thymine substituted 2-aminoethylglycyl units, which C-terminates in a lysine amide and N-terminates in a complex 9-aminoacridine ligand (9-Acr^1-(Taeg)$_{10}$-Lys-NH_2, Figure 11a, 11b) to a dA_{10}/dT_{10} target sequence. The target is contained in a 248 bp ^{32}P-end-labelled DNA-fragment.

Strand displacement was ascertained by the following type of experiments:

1) The 9-Acr^1 ligand (Figure 5), which is equipped with a 4-nitrobenzamido group to ensures cleavage of DNA upon irradiation, is expected only to cleave DNA in close proximity to its binding site. Upon irradiation of the PNA with the above 248 bp DNA fragment, selective cleavage at the dA_{10}/dT_{10} sequence is observed (Figure 3a).

2) In a so-called photofootprinting assay, where a synthetic diazo-linked acridine under irradiation cleaves DNA (except where the DNA is protected by said binding substance) upon interaction with DNA in the presence of a DNA-binding substance.

Such an experiment was performed with the above 248 bp dsDNA fragment, which showed clear protecti n against ph - tocleavage of the PNA binding site (Figure 3b).

3) In a similar type f experiment, the DNA-cleaving enzyme micrococcus nuclease, which is als hindered in its

action by m st DNA-binding reagents, showed increased cleavage at the T_{10}-target (Figure 3c).

4) In yet another type of experiment, the well-known high susceptibility of single strand thymine ligands (as opposed to double strand thymine ligands) towards potassium permanganate oxidation was employed. Oxidation of the 248 bp in the presence of the reagent showed only oxidation of the T_{10}-strand of the target (Figure 3b).

5) In a similar type of demonstration, the single strand specificity of S_1 nuclease clearly showed that only the T_{10}-strand of the target was attacked (Figure 3d).

The very efficient binding of $(Taeg)_{10}$, $(Taeg)_{10}$-Lys-NH_2 and Acr^1-$(Taeg)_{10}$-Lys-NH_2 (Figures 11a. 11b) to the corresponding dA_{10} was furthermore illustrated in two ways:

1. Ligand-oligonucleotide complexes will migrate slower than the naked oligonucleotide upon electrophoresis in polyacrylamide gels. Consequently, such experiments were performed with Acr^1-$(Taeg)_{10}$-Lys-NH_2 and ^{32}P-end-labelled dA_{10}. This showed retarded migration under conditions where a normal dA_{10}/dT_{10} duplex is stable, as well as under conditions where such a duplex is unstable (denaturing gel). A control experiment was performed with a mixture of Acr^1-$(Taeg)_{10}$-Lys-NH_2 and ^{32}P-end-labelled dT_{10} which showed no retardation under the above conditions.

2. Upon formation of DNA duplexes (dsDNA) from single strand DNA, the extinction coefficient decreases (hypochromicity). Thus, the denaturing of DNA can be followed by measuring changes in the absorbance, for example, as a function of T_m, the temperature where 50% of a duplex has disappeared to give single strands.

Duplexes were formed from the single-stranded oligodeoxyribonucleotides and the PNAs listed below. Typically 0.3 OD_{260} of the T-rich strand was hybridized with 1 equivalent of the other strand by heating to 90 C for 5 min, cooling t room temperature and kept for 30 min and finally stor d in a refrigerator at 5 C for at least 30 min. Th buffers us d wer all 10 mM in phosphate and 1 mM in EDTA.

The l w salt buffer c ntained no sodium chlorid , whereas the medium salt buffer c ntained 140 mM NaCl and the high salt buffer 500 mM NaCl. The pH of all the buffers was 7.2. The melting temperature of the hybrids were determined on a Gilford Response apparatus. The following extinction coefficients were used A: 15.4 ml/µmol·cm; T: 8.8; G: 11.7 and C: 7.3 for both normal oligonucleotides and PNA. The melting curves were recorded in steps of 0.5 C/min. The T_m were determined from the maximum of the 1st derivative of the plot of A_{260} vs témperature.

List of oligodeoxyribonucleotides:

1. 5′-AAA-AAA-AA
2. 5′-AAA-AAA-AAA-A
3. 5′-TTT-TTT-TTT-T
4. 5′-AAA-AAG-AAA-A
5. 5′-AAG-AAG-AAA-A
6. 5′-AAA-AGA-AAA-A
7. 5′-AAA-AGA-AGA-A
8. 5′-TTT-TCT-TTT-T
9. 5′-TTT-TCT-TCT-T
10. 5′-TTT-TTC-TTT-T
11. 5′-TTT-TTC-TTC-T
12. 5′-TTC-TTC-TTT-T
13. 5′-TTT-TTT-TTT-TTT-TTT
14. 5′-AAA-AAA-AAA-AAA-AAA

List of PNAs

a. TTT-TTT-TTT-T-Lys-NH_2
b. TTT-TTT-TT-Lys-NH_2
c. TTT-TTC-TTT-T-Lys-NH_2
d. TTC-TTC-TTT-T-Lys-NH_2
e. Acr-TTT-TTT-TTT-T-Lys-NH_2
f. Ac-TTT-TTT-TTT-T-Lys-NH_2

Oligo/PNA	Low Salt	Medium Salt	High Salt
1+b	56.0	51.5	50.0
2+a	73.0	72.5	73.0
2+c		41.5 and 52.0*	
2+e	84.5	86.0	≈90
2+f		74	
4+a	60.0	59.0	61.5
4+c	74.5	72.0	72.5
4+f		62.0	
5+a		47.0	
5+c		57.5	
5+f		46.5	
7+a		46.0	
7+c		58.0	
7+f		43.5	
7+12		23.0	
13+14		39.0	

* = Two distinct melting temperatures are seen, indicating local melting before complet denaturation.

The hybrid formed between RNA-A (poly rA) and PNA-T_{10}-Lys-NH_2 melts at such high temperature that it cannot be measured (>90 C). But specific hybridization is demonstrated by the large drop in A_{260} by mixing with RNA-A but not G,C and U. The experiment is done by mixing 1 ml of a solution of the PNA and 1 ml of a solution the RNA, each with A_{260} = 0.6, and then measure the absorbance at 260 nm. Thereafter the sample is heated to 90 C for 5 min, cooled to room temperature and kept at this temperature for 30 minutes and finally stored at 5C for 30 min.

RNA	PNA	A_{260} Before Mixing	A_{260} After Mixing	A_{260} After Mixing and Heating
RNA-A	PNA-T_{10}lys-NH_2	0.600	0.389	0.360
RNA-U	PNA-T_{10}-lys-NH_2	0.600	0.538	0.528
RNA-G	PNA-T_{10}-lys-NH_2	0.600	0.514	0.517
RNA-C	PNA-T_{10}-lys-NH_2	0.600	0.540	0.532

From the above measurements the following conclusions can be made. There is base stacking, since a melting curve is observed. The PNA-DNA hybrid is more stable than a normal DNA-DNA hybrid, and the PNA-RNA is even more stable. Mismatches cause significant drops in the T_m-value, whether the mispaired base is in the DNA or in the PNA-strand. The T_m-value is only slightly dependent on ionic strength, as opposed to normal oligonucleotides.

The synthesis of the PNAs according to the invention is discussed in detail in the following, where Figure 1 illustrates one of the preferred PNA examples and compares its structure to that of a complementary DNA.

Synthesis of PNA Oligomers and Polymers

The principle of anchoring molecules onto a solid matrix, which helps in accounting for intermediate products during chemical transformations, is known as Solid-Phase Synthesis or Merrifield Synthesis (*see, e.g.*, Merrifield, *J. Am. Chem. Soc.*, 1963, *85*, 2149 and *Science*, 1986, *232*, 341). Established methods for the stepwise or fragmentwise solid-phase assembly of amino acids into peptides normally employ a beaded matrix of slightly cross-linked styrene-divinylbenzene copolymer, the cross-linked copolymer having been formed by the pearl polymerization of styrene monomer to which has been added a mixture of divinylbenzenes. A lev 1 of 1-2% cross-linking is usually employed. Such a matrix als can be used in solid-phase PNA synthesis in accordance with the present invention (Figure 8).

Concerning the initial functionalization f the solid phase, more than fifty methods have been d scribed in connection with traditional solid-phase peptide synthesis (*see, e.g.*, Barany and Merrifield in "The Peptides" Vol. 2, Academic Press, New York, 1979, pp. 1-284, and Stewart and Young, "Solid Phase Peptide Synthesis", 2nd Ed., Pierce Chemical Company, Illinois, 1984). Reactions for the introduction of chloromethyl functionality (Merrifield resin; via a chloromethyl methyl ether/$SnCl_4$ reaction), aminomethyl functionality (via an N-hydroxymethylphthalimide reaction; *see*, Mitchell, *et al.*, *Tetrahedron Lett.*, 1976, *3795*), and benzhydrylamino functionality (Pietta, *et al.*, *J. Chem. Soc.*, 1970, 650) are the most widely applied. Regardless of its nature, the purpose of the functionality is normally to form an anchoring linkage between the copolymer solid support and the C-terminus of the first amino acid to be coupled to th solid support. As will be recognized, anchoring linkages also can be formed between the solid support and the amino acid N-terminus. It is generally convenient to express the "concentration" of a functional group in terms of millimoles per gram (mmol/g). Other reactive functionalities which have been initially introduced include 4-methylbenzhydrylamino and 4-methoxybenzhydrylamino. All of these established methods are in principle useful within the context of the present invention. Preferred methods for PNA synthesis employ aminomethyl as the initial functionality, in that aminomethyl is particularly advantageous with respect to the incorporation of "spacer" or "handle" groups, owing to the reactivity of the amino group of the aminomethyl functionality with respect t the essentially quantitative formation of amide bonds to a carboxylic acid group at one end of the spacer-forming reagent. A vast number of relevant spacer- or handle-forming bifunctional reagents have been described (*see*, Barany, *et al.*, *Int. J. Peptide Protein Res.*, 1987, *30*, 705), especially r agents which are reactive towards amino groups such as f und in the aminomethyl function. Representative bifunctional r agents include 4-(haloalkyl)aryl-l wer alkanoic acids such

as 4-(brom methyl)phenylacetic acid, Boc-aminoacyl-4-(oxym thyl)aryl-lower alkan ic acids such as Boc-aminoacyl-4-(oxymethyl)phenylacetic acid, N-Boc-p-acylbenzhydrylamines such as N-Boc-p-glutaroylbenzhydrylamine, N-Boc-4'-lower alkyl-p-acylbenzhydrylamines such as N-Boc-4'-methyl-p-glutaroylbenzhydrylamine, N-Boc-4'-lower alkoxy-p-acylbenzhydrylamines such as N-Boc-4'-methoxy-p-glutaroyl-benzhydrylamine, and 4-hydroxymethylphenoxyacetic acid. One typ of spacer group particularly relevant within the context of the present invention is the phenylacetamidomethyl (Pam) handle (Mitchell and Merrifield, *J. Org. Chem.*, 1976, *41*, 2015) which, deriving from the electron withdrawing effect of the 4-phenylacetamidomethyl group, is about 100 times mor stable than the classical benzyl ester linkage towards the Boc-amino deprotection reagent trifluoroacetic acid (TFA).

Certain functionalities (*e.g.*, benzhydrylamino, 4-methylbenzhydrylamino and 4-methoxybenzhydrylamino) which may be incorporated for the purpose of cleavage of a synthesized PNA chain from the solid support such that the C-terminal of the PNA chain is in amide form, require no introduction of a spacer group. Any such functionality may advantageously be employed in the context of the present invention.

An alternative strategy concerning the introduction of spacer or handle groups is the so-called "preformed handle" strategy (*see*, Tam, *et al.*, *Synthesis*, 1979, 955-957), which offers complete control over coupling of the first amino acid, and excludes the possibility of complications arising from the presence of undesired functional groups not related to the peptide or PNA synthesis. In this strategy, spacer or handle groups, of the same type as described above, are reacted with the first amino acid desired to be bound to the solid support, the amino acid being N-protected and optionally protected at the other side-chains which are not relevant with respect to the growth of the desired PNA chain. Thus, in those cases in which a spacer r handle group is desirable, the first amin acid to be coupl d to the solid supp rt can either be coupled to the free reactive end f a spacer group which has been

b und to the initially introduced functi nality (f r example, an aminom thyl group) or can be r acted with the spacer-f rming reagent. The space-forming reagent is then reacted with the initially introduced functionality. Other useful anchoring schemes include the "multidetachable" resins (Tam, *et al.*, *Tetrahedron Lett.*, 1979, 4935 and *J. Am. Chem. Soc.*, 1980, *102*, 611; Tam, *J. Org. Chem.*, 1985, *50*, 5291), which provide more than one mode of release and thereby allow more flexibility in synthetic design.

Suitable choices for N-protection are the tert-butyloxycarbonyl (Boc) group (Carpino, *J. Am. Chem. Soc.*, 1957, *79*, 4427; McKay, *et al.*, *J. Am. Chem. Soc.*, 1957, *79*, 4686; Anderson, *et al.*, *J. Am. Chem. Soc.*, 1957, *79*, 6180) normally in combination with benzyl-based groups for the protection of side chains, and the 9-fluorenylmethyloxy-carbonyl (Fmoc) group (Carpino, *et al.*, *J. Am. Chem. Soc.*, 1970, *92*, 5748 and *J. Org. Chem.*, 1972, *37*, 3404), normally in combination with tert-butyl (tBu) for the protection of any side chains, although a number of other possibilities exist which are well known in conventional solid-phase peptid synthesis. Thus, a wide range of other useful amin protecting groups exist, some of which are Adoc (Hass, *et al.*, *J. Am. Chem. Soc.*, 1966, *88*, 1988), Bpoc (Sieber, *Helv. Chem. Acta.*, 1968, *51*, 614), Mcb (Brady, *et al.*, *J. Org. Chem.*, 1977, *42*, 143), Bic (Kemp, *et al.*, *Tetrahedron*, 1975, 4624), the o-nitrophenylsulfenyl (Nps) (Zervas, *et al.*, *J. Am. Chem. Soc.*, 1963, *85*, 3660), and the dithiasuccinoyl (Dts) (Barany, *et al.*, *J. Am. Chem. Soc.*, 1977, *99*, 7363). These amino protecting groups, particularly those based on the widely-used urethane functionality, successfully prohibit racemization (mediated by tautomerization of the readily formed oxazolinone (azlactone) intermediates (Goodman, *et al.*, *J. Am. Chem. Soc.*, 1964, *86*, 2918)) during the coupling of most α-amino acids. In addition to such amino protecting groups, a whole rang of otherwise "worthless" n nurethane-type f amino pr tecting gr ups are applicable when assembling PNA molecules,

esp cially those built from achiral units. Thus, not only the above-mentioned amin protecting groups (or those derived fr m any of these groups) are useful within the context of the present invention, but virtually any amino protecting group which largely fulfills the following requirements: (1) stability to mild acids (not significantly attacked by carboxyl groups); (2) stability to mild bases or nucleophiles (not significantly attacked by the amino group in question); (3) resistance to acylation (not significantly attacked by activated amino acids). Additionally: (4) the protecting group must be close to quantitatively removable, without serious side reactions, and (5) the optical integrity, if any, of the incoming amino acid should preferably be highly preserved upon coupling. Finally, the choice of side-chain protecting groups, in general, depends on the choice of the amino protecting group, since the protection of side-chain functionalities must withstand the conditions of the repeated amino deprotection cycles. This is true whether the overall strategy for chemically assembling PNA molecules relies on, for example, differential acid stability of amino and side-chain protecting groups (such as is the case for the above-mentioned "Boc-benzyl" approach) or employs an orthogonal, that is, chemoselective, protection scheme (such as is the case for the above-mentioned "Fmoc-tBu" approach),

Following coupling of the first amino acid, the next stage of solid-phase synthesis is the systematic elaboration of the desired PNA chain. This elaboration involves repeated deprotection/coupling cycles. The temporary protecting group, such as a Boc or Fmoc group, on the last-coupled amino acid is quantitatively removed by a suitable treatment, for example, by acidolysis, such as with trifluoroacetic acid, in the case of Boc, or by base treatment, such as with piperidine, in the case of Fmoc, so as to liberate the N-terminal amine function.

The next desired N-protected amin acid is then coupled to the N-terminal of the last-c upled amino acid. This coupling of the C-terminal of an amino acid with the N-

t rminal f the last-coupled amino acid can be achieved in several ways. For example, th carboxyl gr up of th inc ming amino acid can be reacted directly with the N-terminal of the last-coupled amino acid with the assistance of a condensation reagent such as, for example, dicyclohexylcarbodiimide (DCC) (Sheehan & Hess, *et al.*, *J. Am. Chem. Soc.*, 1955, *77*, 1067) and diisoproplycarbodiimide (DIC) (Sraantakis et al., *Biochem. biophys. res. Commun.*, 1976, 73, 336) or derivatives thereof. Alternatively, it can be bound by providing the incoming amino acid in a form with the carboxyl group activated by any of several methods, including the initial formation of an active ester derivative such as a 2,4,5-trichlorophenyl ester (Pless, *et al.*, *Helv. Chim. Acta*, 1963, *46*, 1609), a phthalimido ester (Nefkens, *et al.*, *J. Am. Chem. Soc.*, 1961, *83*, 1263), a pentachlorophenyl ester (Kupryszewski, *Rocz. Chem.*, 1961, *35*, 595), a pentafluorophenyl ester (Kovacs, *et al.*, *J. Am. Chem. Soc.*, 1963, *85*, 183), an o-nitrophenyl ester (Bodanzsky, *Nature*, 1955, *175*, 685), an imidazole ester (Li, *et al.*, *J. Am. Chem. Soc.*, 1970, *92*, 7608), and a 3-hydroxy-4-oxo-3,4-dihydroquinazoline (Dhbt-OH) ester (Konig, *et al.*, *Chem. Ber.*, 1973, *103*, 2024 and 2034), or the initial formation of an anhydride such as a symmetrical anhydride (Wieland, *et al.*, *Angew. Chem., Int. Ed. Engl.*, 1971, *10*, 336). Benzotriazolyl N-oxytrisdimethylaminophosphonium hexafluorophosphate (BOP), "Castro's reagent" (*see, e.g.*, Rivaille, *et al.*, *Tetrahedron*, 1980, *36*, 3413) is recommended when assembling PNA molecules containing secondary amino groups. Finally, activated PNA monomers analogous to the recently-reported amino acid fluorides (Carpino, *J. Am. Chem. Soc.*, 1990, *112*, 9651) hold considerable promise to be used in PNA synthesis as well.

Following assembly of the desired PNA chain, including protecting groups, the next step will normally be deprotection of the amino acid moieties of the PNA chain and cleavage of th synthesized PNA from the solid supp rt. These pr cesses can take place substantially simultaneously, thereby providing the fr e PNA molecule in the desired form. Alternatively, in cases in which condensation of two separately synthesized PNA

chains is to be carried out, it is p ssible by ch osing a suitable spacer group at th start of th synth sis to cleave the desired PNA chains from their respective solid supports (both peptide chains still incorporating their side-chain protecting groups) and finally removing the side-chain protecting groups after, for example, coupling the two side-chain protected peptide chains to form a longer PNA chain.

In the above-mentioned "Boc-benzyl" protection scheme, the final deprotection of side-chains and release of the PNA molecule from the solid support is most often carried out by the use of strong acids such as anhydrous HF (Sakakibara, *et al.*, *Bull. Chem. Soc. Jpn.*, 1965, *38*, 4921), boron tris (trifluoroacetate) (Pless, *et al.*, *Helv. Chim. Acta*, 1973, *46*, 1609), and sulfonic acids such as trifluoromethanesulfonic acid and methanesulfonic acid (Yajima, et *al.*, *J. Chem. Soc., Chem. Comm.*, 1974, 107). This conventional strong acid (*e.g.*, anhydrous HF) deprotection method, produces very reactive carbocations that may lead to alkylation and acylation of sensitive residues in the PNA chain. Such side-reactions are only partly avoided by the presence of scavengers such as anisole, phenol, dimethyl sulfide, and mercaptoethanol and, therefore, the sulfide-assisted acidolytic S_N2 deprotection method (Tam, et *al.*, *J. Am. Chem. Soc.*, 1983, *105*, 6442 and *J. Am. Chem. Soc.*, 1986, *108*, 5242), the so-called "low", which removes the precursors of harmful carbocations to form inert sulfonium salts, is frequently employed in peptide and PNA synthesis, either solely or in combination with "high" methods. Less frequently, in special cases, other methods used for deprotection and/or final cleavage of the PNA-solid support bond are, for example, such methods as base-catalyzed alcoholysis (Barton, *et al.*, *J. Am. Chem. Soc.*, 1973, *95*, 4501), and ammonolysis as well as hydrazinolysis (Bodanszky, *et al.*, *Chem. Ind.*, 1964 1423), hydrogenolysis (Jones, *Tetrahedron Lett.* 1977 2853 and Schlatter, *et al.*, *Tetrahedron Lett.* 1977 2861)), and ph tolysis (Rich and Gurwara, *J. Am. Chem. Soc.*, 1975 *97*, 1575)).

Finally, in c ntrast with the chemical synthesis of "normal" peptides, stepwise chain building of achiral PNAs such as those based on aminoethylglycyl backbone units can start either from the N-terminus or the C-terminus, because the coupling reactions are free of racemization. Those skilled in the art will recognize that whereas syntheses commencing at the C-terminus typically employ protected amine groups and free or activated acid groups, syntheses commencing at the N-terminus typically employ protected acid groups and free or activated amine groups.

Based on the recognition that most operations are identical in the synthetic cycles of solid-phase peptide synthesis (as is also the case for solid-phase PNA synthesis), a new matrix, PEPS, was recently introduced (Berg, *et al.*, *J. Am. Chem. Soc.*, 1989, *111*, 8024 and International Patent Application WO 90/02749) to facilitate the preparation of large numbers of peptides. This matrix is comprised of a polyethylene (PE) film with pendant long-chain polystyrene (PS) grafts (molecular weight on the order of 10^6). The loading capacity of the film is as high as that of a beaded matrix, but PEPS has the additional flexibility to suit multiple syntheses simultaneously. Thus, in a new configuration for solid-phase peptide synthesis, the PEPS film is fashioned in the form of discrete, labeled sheets, each serving as an individual compartment. During all the identical steps of the synthetic cycles, the sheets are kept together in a single reaction vessel to permit concurrent preparation of a multitude of peptides at a rate close to that of a single peptide by conventional methods. It was reasoned that the PEPS film support, comprising linker or spacer groups adapted to the particular chemistry in question, should be particularly valuable in the synthesis of multiple PNA molecules, these being conceptually simple to synthesize since only four different reaction compartments are normally required, one for each of the four "pseud -nucle tide" units. Thus, the PEPS film support has b n successfully tested in a number of PNA syntheses carried ut in a parallel and

substantially simultan ous fashi n. Th yi ld and quality of the products obtained fr m PEPS wer comparable t th se obtained by using the traditional polystyrene beaded support. Also, experiments with other geometries of the PEPS polymer such as, for example, non-woven felt, knitted net, sticks or microwellplates have not indicated any limitations of the synthetic efficacy.

Two other methods proposed for the simultaneous synthesis of large numbers of peptides also apply to the preparation of multiple, different PNA molecules. The first of these methods (Geysen, *et al.*, *Proc. Natl. Acad. Sci. USA*, 1984, *81*, 3998) utilizes acrylic acid-grafted polyethylene-rods and 96-microtiter wells to immobilize the growing peptide chains and to perform the compartmentalized synthesis. While highly effective, the method is only applicable on a microgram scale. The second method (Houghten, *Proc. Natl. Acad. Sci. USA*, 1985, *82*, 5131) utilizes a "tea bag" containing traditionally-used polymer beads. Other relevant proposals for multiple peptide or PNA synthesis in the context of the present invention include the simultaneous use of two different supports with different densities (Tregear, in "*Chemistry and Biology of Peptides*", J. Meienhofer, ed., Ann Arbor Sci. Publ., Ann Arbor, 1972 pp. 175-178), combining of reaction vessels via a manifold (Gorman, *Anal. Biochem.*, 1984, *136*, 397), multicolumn solid-phase synthesis (e.g. Krchnak, *et al.*, *Int. J. Peptide Protein Res.*, 1989, *33*, 209), and Holm and Meldal, in "*Proceedings of the 20th European Peptide Symposium*", G. Jung and E. Bayer, eds., Walter de Gruyter & Co., Berlin, 1989 pp. 208-210), and the use of cellulose paper (Eichler, *et al.*, *Collect. Czech. Chem. Commun.*, 1989, *54*, 1746).

While the conventional cross-linked styrene/divinylbenzene copolymer matrix and the PEPS support are presently preferred in the context f solid-phase PNA synthesis, a non-limiting list of examples f solid supp rts which may be of relevance are: (1) Particles based up n copolymers of dimethylacrylamide cr ss-linked with N,N'-

bisacryl ylethylenediamin , including a known am unt f N-t rtbutoxycarb nyl-beta-alanyl-N'-acryl ylhexamethylenediamine. Several spacer molecules are typically added via the beta alanyl group, followed thereafter by the amino acid residue subunits. Also, the beta alanyl-containing monomer can be replaced with an acryloyl sarcosine monomer during polymerization to form resin beads. Th polymerization is followed by reaction of the beads with ethylenediamine to form resin particles that contain primary amines as the covalently linked functionality. The polyacrylamide-based supports are relatively more hydrophilic than are the polystyrene-based supports and are usually used with polar aprotic solvents including dimethylformamide, dimethylacetamide, N-methylpyrrolidone and the like (see Atherton, *et al.*, *J. Am. Chem. Soc.*, 1975, *97*, 6584, *Bioorg. Chem.* 1979, *8*, 351), and J.C.S. Perkin I 538 (1981)); (2) a second group of solid supports is based on silica-containing particles such as porous glass beads and silica gel. One example is the reaction product of trichloro-[3-(4-chloromethyl)phenyl]propylsilane and porous glass beads (se Parr and Grohmann, *Angew. Chem. Internal. Ed.* 1972, *11*, 314) sold under the trademark "PORASIL E" by Waters Associates, Framingham, MA, USA. Similarly, a mono ester of 1,4-dihydroxymethylbenzene and silica (sold under the trademark "BIOPAK" by Waters Associates) has been reported to be useful (se Bayer and Jung, *Tetrahedron Lett.*, 1970, 4503); (3) a third general type of useful solid supports can be termed composites in that they contain two major ingredients: a resin and another material that is also substantially inert to the organic synthesis reaction conditions employed. One exemplary composite (see Scott, *et al.*, *J. Chrom. Sci.*, 1971, *9*, 577) utilized glass particles coated with a hydrophobic, cross-linked styrene polymer containing reactive chloromethyl groups, and was supplied by Northgate Laboratories, Inc., of Hamden, CT, USA. An ther exemplary composite contains a c re of fluorinated ethylen polymer onto which has be n grafted p lystyren (see Kent and Merrifield, *Israel J. Chem.* 1978,

17, 243) and van Rietsch ten in "*Peptides 1974*", Y. Wolman, Ed., Wiley and S ns, New York, 1975, pp. 113-116); and (4) contiguous solid supports other than PEPS, such as cotton sheets (Lebl and Eichler, *Peptide Res.* 1989, *2*, 232) and hydroxypropylacrylate-coated polypropylene membranes (Daniels, *et al.*, *Tetrahedron Lett.* 1989, 4345), are suited for PNA synthesis as well.

Whether manually or automatically operated, solid-phase PNA synthesis in the context of the present invention is normally performed batchwise. However, most of the syntheses may equally well be carried out in the continuous-flow mode, where the support is packed into columns (Bayer, *et al.*, *Tetrahedron Lett.*, 1970, 4503 and Scott, *et al.*, *J. Chromatogr. Sci.*, 1971, *9*, 577). With respect to continuous-flow solid-phase synthesis, the rigid poly(dimethylacrylamide)-Kieselguhr support (Atherton, *et al.*, *J. Chem. Soc. Chem. Commun.*, 1981, 1151) appears to be particularly successful, but another valuable configuration concerns the one worked out for the standard copoly(styrene-1%-divinylbenzene) support (Krchnak, *et al.*, *Tetrahedron Lett.*, 1987, 4469).

While the solid-phase technique is presently preferred in the context of PNA synthesis, other methodologies or combinations thereof, for example, in combination with the solid-phase technique, apply as well: (1) the classical solution-phase methods for peptide synthesis (*e.g.*, Bodanszky, "*Principles of Peptide Synthesis*", Springer-Verlag, Berlin-New York 1984), either by stepwise assembly or by segment/fragment condensation, are of particular relevance when considering especially large scale productions (gram, kilogram, and even tons) of PNA compounds; (2) the so-called "liquid-phase" strategy, which utilizes soluble polymeric supports such as linear polystyrene (Shemyakin, *et al.*, *Tetrahedron Lett.*, 1965, 2323) and polyethylene glycol (PEG) (Mutter and Bayer, *Angew. Chem., Int. Ed. Engl.*, 1974, *13*, 88), is useful; (3) random polymerization (*see*, *e.g.*, Odian, "*Principl s of Polymerization*", McGraw-Hill, New York (1970)) yielding

mixtures of many molecular weights ("p lydisperse") peptide r PNA m lecules are particularly relevant f r purposes such as screening for antiviral effects; (4) a technique based on the use of polymer-supported amino acid active esters (Fridkin, *et al.*, *J. Am. Chem. Soc.*, 1965, *87*, 4646), sometimes referred to as "inverse Merrifield synthesis" or "polymeric reagent synthesis", offers the advantage of isolation and purification of intermediate products, and may thus provide a particularly suitable method for the synthesis of medium-sized, optionally protected, PNA molecules, that can subsequently be used for fragment condensation into larger PNA molecules; (5) it is envisaged that PNA molecules may be assembled enzymatically by enzymes such as proteases or derivatives thereof with novel specificities (obtained, for example, by artificial means such as protein engineering). Also, one can envision the development of "PNA ligases" for the condensation of a number of PNA fragments into very large PNA molecules; (6) since antibodies can be generated to virtually any molecule of interest, the recently developed catalytic antibodies (abzymes), discovered simultaneously by the groups of Lerner (Tramantano, *et al.*, *Science*, 1986, *234*, 1566) and of Schultz (Pollack, *et al.*, *Science*, 1986, *234*, 1570), should also be considered as potential candidates for assembling PNA molecules. Thus, there has been considerable success in producing abzymes catalyzing acyl-transfer reactions (see for example Shokat, *et al.*, *Nature*, 1989, *338*, 269) and references therein). Finally, completely artificial enzymes, very recently pioneered by Stewart's group (Hahn, *et al.*, *Science*, 1990, *248*, 1544), may be developed to suit PNA synthesis. The design of generally applicable enzymes, ligases, and catalytic antibodies, capable of mediating specific coupling reactions, should be more readily achieved for PNA synthesis than for "normal" peptide synthesis since PNA molecules will often be comprised of only four different amino acids (one f r each of the four native nucleobases) as c mpared t the twenty natural by occurring (proteinog nic) amino acids constituting p ptides. In conclusion, no single

strategy may be wholly suitable for the synthesis of a specific PNA mol cule, and therefore, sometimes a combinati n of methods may work best.

The present invention also is directed to therapeutic or prophylactic uses for peptide nucleic acids. Likely therapeutic and prophylactic targets include herpes simplex virus (HSV), human papillomavirus (HPV), human immunodeficiency virus (HIV), candidia albicans, influenza virus, cytomegalovirus (CMV), intracellular adhesion molecules (ICAM), 5-lipoxygenase (5-LO), phospholipase A_2 (PLA_2), protein kinase C (PKC), and RAS oncogene. Potential applications of such targeting include treatments for ocular, labial, genital, and systemic herpes simplex I and II infections; genital warts; cervical cancer; common warts; Kaposi's sarcoma; AIDS; skin and systemic fungal infections; flu; pneumonia; retinitis and pneumonitis in immunosuppressed patients; mononucleosis; ocular, skin and systemic inflammation; cardiovascular disease; cancer; asthma; psoriasis; cardiovascular collapse; cardiac infarction; gastrointestinal disease; kidney disease; rheumatoid arthritis; osteoarthritis; acute pancreatitis; septic shock; and Crohn's disease.

For therapeutic or prophylactic treatment, the peptide nucleic acids of the invention can be formulated in a pharmaceutical composition, which may include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like in addition to peptide nucleic acid.

The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administrati n may b done topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalati n, or parenterally, f r example by intravenous

drip or subcutan us, intraperit n al or intramuscular injection.

F rmulations f r topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and th like may be necessary or desirable. Coated condoms may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable.

Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

Dosing is dependent on severity and responsiveness of the condition to be treated, but will normally be one or mor doses per day, with course of treatment lasting from several days to several months or until a cure is effected or a diminution of disease state is achieved. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates.

Treatments of this type can be practiced one a variety of organisms ranging from unicellular prokaryotic and eukaryotic organisms to multicellular eukaryotic organisms. Any organism that utilizes DNA-RNA transcription or RNA-protein translation as a fundamental part of its hereditary, metabolic or cellular control is susceptible to therapeutic and/or prophylactic treatment in accordance with the invention. Seemingly diverse organisms such as bacteria, yeast, protozoa, algae, all plants and all higher animal forms, including warm-blooded animals, can be treated. Further, since each cell of multicellular eukaryotes can be treated since they include both DNA-RNA transcription and RNA-pr tein translation as integral parts f their c llular activity. Furthermor , many of the organelles (*e.g.*, mitochondria and chloroplasts) f

eukaryotic cells als include transcription and translation mechanisms. Thus, singl c lls, cellular populations r organelles can also be included within the definition of organisms that can be treated with therapeutic or diagnostic phosphorothioate oligonucleotides. As used herein, therapeutics is meant to include the eradication of a disease state, by killing an organism or by control of erratic or harmful cellular growth or expression.

The present invention also pertains to the advantageous use of PNA molecules in solid-phase biochemistry (*see, e.g.*, "*Solid-Phase Biochemistry - Analytical and Synthetic Aspects*", W. H. Scouten, ed., John Wiley & Sons, New York, 1983), notably solid-phase biosystems, especially bioassays or solid-phase techniques which concerns diagnostic detection/quantitation or affinity purification of complementary nucleic acids (*see, e.g.*, "*Affinity Chromatography - A Practical Approach*", P. D. G. Dean, W. S. Johnson and F. A. Middle, eds., IRL Press Ltd., Oxford 1986; "*Nucleic Acid Hybridization - A Practical Approach*", B. D. Harnes and S. J. Higgins, IRL Press Ltd., Oxford 1987). Present day methods for performing such bioassays or purification techniques almost exclusively utilize "normal" or slightly modified oligonucleotides either physically adsorbed or bound through a substantially permanent covalent anchoring linkage to beaded solid supports such as cellulose, glass beads, including those with controlled porosity (Mizutani, *et al.*, *J. Chromatogr.*, 1986, *356*, 202), "Sephadex", "Sepharose", agarose, polyacrylamide, porous particulate alumina, hydroxyalkyl methacrylate gels, diol-bonded silica, porous ceramics, or contiguous-materials such as filter discs of nylon and nitrocellulose. One example employed the chemical synthesis of oligo-dT on cellulose beads for the affinity isolation of poly A tail containing mRNA (Gilham in "*Methods in Enzymology*," L. Grossmann and K. Moldave, eds., v l. 21, part D, page 191, Academic Press, New York and L ndon, 1971). All the above-mentioned methods are applicable within the context of the present inventi n. H wever, when p ssible, covalent linkage is preferred over the

physical adsorption of the mol cules in question, since the latter appr ach has the disadvantage that som of the immobilized molecules can be washed out (desorbed) during the hybridization or affinity process. There is, thus, littl control of the extent to which a species adsorbed on the surface of the support material is lost during the various treatments to which the support is subjected in the course of the bioassay/purification procedure. The severity of this problem will, of course, depend to a large extent on the rate at which equilibrium between adsorbed and "free" species is established. In certain cases it may be virtually impossible to perform a quantitative assay with acceptable accuracy and/or reproducibility. Loss of adsorbed species during treatment of the support with body fluids, aqueous reagents or washing media will, in general, be expected to be most pronounced for species of relatively low molecular weight. In contrast with oligonucleotides, PNA molecules are easier to attach onto solid supports because they contain strong nucleophilic and/or electrophilic centers. In addition, the direct assembly of oligonucleotides onto solid supports suffers from an extremely low loading of the immobilized molecule, mainly due to the low surface capacity of the materials that allow the successful use of the state-of-the-art phosphoramidite chemistry for the construction of oligonucleotides. (Beaucage and Caruthers, *Tetrahedron Lett.*, 1981, *22*, 1859; Caruthers, *Science*, 1985, *232*, 281). It also suffers from the fact that by using the alternative phosphite triester method (Letsinger and Mahadevan, *J. Am. Chem. Soc.* 1976, *98*, 3655), which is suited for solid supports with a high surface/loading capacity, only relatively short oligonucleotides can be obtained. As for conventional solid-phase peptide synthesis, however, the latter supports are excellent materials for building up immobilized PNA molecules (the side-chain protecting groups are removed from the synthesized PNA chain without cleaving the anchoring linkag h lding the chain t the s lid support). Thus,. PNA species benefit from the above-described solid-phase techniques with respect to the

much higher (and still sequenc -specific) binding affinity for c mplementary nucleic acids and from the additi nal unique sequence-specific recognition of (and strong binding to) nucleic acids present in double-stranded structures. They also can be loaded onto solid supports in large amounts, thus further increasing the sensitivity/capacity of the solid-phase technique. Further, certain types of studies concerning the use of PNA in solid-phase biochemistry can be approached, facilitated, or greatly accelerated by use of the recently-reported "light-directed, spatially addressable, parallel chemical synthesis" technology (Fodor, *et al.*, *Science*, 1991, *251*, 767), a technique that combines solid-phase chemistry and photolithography to produce thousands of highly diverse, but identifiable, permanently immobilized compounds (such as peptides) in a substantially simultaneous way.

Additional objects, advantages, and novel features of this invention will become apparent to those skilled in the art upon examination of the following examples thereof, which are not intended to be limiting.

Synthesis of monomeric building blocks

The monomers preferably are synthesized by the general scheme outlined in Figure 13. This involves preparation of either the methyl or ethyl ester of (Bocaminoethyl)glycine, by a protection/deprotection procedure as described in Examples 24-26. The synthesis of thymine monomer is described in Examples 27-28, and that of the protected cytosine monomer is described in Example 29.

The synthesis of the protected adenine monomer (Figure 14) involved alkylation with ethyl bromoacetate (Example 30) and verification of the position of substitution by X-ray crystallography, as being the wanted 9-position. The N^6-amino group then was protected with the benzyloxycarbonyl gr up by the use of the reagent N-ethyl-benzyloxycarbonylimidaz le tetrafluoroborate (Example 31). Simple hydrolysis of the product ester (Example 32) gav N^6-benzyloxycarbonyl-9-

carboxymethyl adenine, which then was us d in the standard procedure (Exampl s 33-34, Figure 13). The adenine monomer has been built into two different PNA-oligomers (Examples 56, 57, 71 and 73).

The synthesis of the protected G-monomer is outlined in Figure 15. The starting material, 2-amino-6-chloropurine, was alkylated with bromoacetic acid (Example 35) and the chlorine atom was then substitutdd with a benzyloxy group (Example 36). The resulting acid was coupled to th (bocaminoethyl) glycine methyl ester (from Example 26) with agent PyBrop™, and the resulting ester was hydrolysed (Example 37). The O^6-benzyl group was removed in the final HF-cleavag step in the synthesis of the PNA-oligomer. Cleavage was verified by finding the expected mass of the final PNA-oligomer, upon incorporation into an PNA-oligomer using diisopropyl carbodiimide as the condensation agent (Examples 55 and 71).

Extended Backbones

Alterations of the groups A, C and D (figure 16) is demonstrated by the synthesis of monomeric building blocks and incorporation into PNA-oligomers.

In one example, the C group was a $CH(CH_3)$ group. The synthesis of the corresponding monomer is outlined in Figure 17. It involves preparation of Boc-protected 1-amino-2,3-propanediol (Example 38), which is cleaved by periodate to give bocaminoacetaldehyde, which is used directly in the next reaction. The bocaminoacetaldehyde can be condensed with a variety of amines; in Example 39, alanine ethyl ester was used. In Examples 40-42, the corresponding thymine monomers were prepared. The monomer has been incorporated into an 8-mer (Example 60) by the DCC-coupling protocol (Examples 56 and 57).

In another example, the D group is a $(CH_2)_3$ group. The synthesis of the corresponding monomer is outlined in figure 18.A and described in Examples 43-44.

In another exampl , th A group is a $(CH_2)_2CO$ gr up. The synthesis of the c rresponding thymine mon mer is outlined figure 18.B and Examples 46 through 48.

In yet another example, the C group is a $(CH_2)_2$ group. The synthesis of the thymine and protected cytosine monomer is outlined in Figure 19 and Examples 49 through 54. Hybridization experiments with a PNA-oligomer containing one unit is described in Examples 61 and 81, which shows a significant lowering of affinity but a retention of specificity.

General Remarks

The following abbreviations are used in the experimental examples: DMF, N,N-dimethylformamide; DCC, N,N-dicyclohexyl carbodiimide; DCU, N,N-dicyclohexyl urea; THF, tetrahydrofuran; aeg, N-acetyl (2'-aminoethyl)glycine; pfp, pentafluorophenyl; Boc, *tert*-butoxycarbonyl; Z, benzyloxy-carbonyl; NMR, nuclear magnetic resonance; s, singlet; d, doublet; dd, doublet of doublets; t; triplet; q, quartet; m, multiplet; b, broad; δ, chemical shift;

NMR spectra were recorded on either a JEOL FX 90Q spectrometer, or a Bruker 250 MHz with tetramethylsilane as internal standard. Mass spectrometry was performed on a MassLab VG 12-250 quadropole instrument fitted with a VG FAB source and probe. Melting points were recorded on Buchi melting point apparatus and are uncorrected. N,N-Dimethylformamide was dried over 4 Å molecular sieves, distilled and stored over 4 Å molecular sieves. Pyridine (HPLC quality) was dried and stored over 4 Å molecular sieves. Other solvents used were either the highest quality obtainable or were distilled before use. Dioxane was passed through basic alumina prior to use. Bocanhydride, 4-nitrophenol, methyl bromoacetate, benzyloxycarbonyl chloride, pentafluorophenol were all obtained through Aldrich Chemical Company. Thymine, cytosine, adenin wer all obtained through Sigma.

Thin layer chromat graphy (Tlc) was performed using the foll wing solvent systems: (1) chlor form:triethyl

amin :methanol, 7:1:2; (2) methylen chloride:methanol, 9:1; (3) chl roform:methan l:acetic acid 85:10:5. Sp ts were visualized by UV (254 nm) or/and spraying with a ninhydrin solution (3 g ninhydrin in 1000 ml 1-butanol and 30 ml acetic acid), after heating at 120°C for 5 min and, after spraying, heating again.

EXAMPLE 1

***tert*-Butyl 4-nitrophenyl carbonate**

Sodium carbonate (29.14 g; 0.275 mol) and 4-nitrophenol (12.75 g; 91.6 mmol) were mixed with dioxane (250 ml). Boc-anhydride (20.0 g; 91.6 mmol) was transferred to the mixture with dioxane (50 ml). The mixture was refluxed for 1 h, cooled to 0°C, filtered and concentrated to 1/3, and then poured into water (350 ml) at 0°C. After stirring for 1/2 h., the product was collected by filtration, washed with water, and then dried over sicapent, *in vacuo*. Yield 21.3 g (97%). M.p. 73.0-74.5°C (litt. 78.5-79.5°C). Anal. for $C_{11}H_{13}NO_5$ found(calc.) C: 55.20(55.23) H: 5.61(5.48) N: 5.82(5.85).

EXAMPLE 2

(N′-Boc-2′-aminoethyl)glycine (2)

The title compound was prepared by a modification of the procedure by Heimer, *et al. Int. J. Pept.*, 1984, *23*, 203-211 N-(2-Aminoethyl)glycine (1, 3.00 g; 25.4 mmol) was dissolved in water (50 ml), dioxane (50 ml) was added, and the pH was adjusted to 11.2 with 2 N sodium hydroxide. *tert*-Butyl-4-nitrophenyl carbonate (7.29 g; 30.5 mmol) was dissolved in dioxane (40 ml) and added dropwise over a period of 2 h, during which time the pH was maintained at 11.2 with 2 N sodium hydroxide. The pH was adjusted periodically to 11.2 for three more hours and then the solution was left overnight. The solution was cooled to 0°C and the pH was carefully adjusted to 3.5 with 0.5 M hydrochloric acid. The aque us solution was washed with chlor form (3 x 200 ml), the pH adjust d to 9.5 with 2N s dium hydr xide and the solution was evaporated to dryness, *in vacuo* (14 mmHg). The residue

was extracted with DMF (25+2x10 ml) and the extracts filtered to remove excess salt. This results in a solution of the title compound in about 60% yield and greater than 95% purity by tlc (system 1 and visualised with ninhydrin, Rf=0.3). Th solution was used in the following preparations of Boc-aeg derivates without further purification.

EXAMPLE 3

N-1-Carboxymethylthymine (4)

This procedure is different from the literature synthesis, but is easier, gives higher yields, and leaves no unreacted thymine in the product. To a suspension of thymine (3, 40.0 g; 0.317 mol) and potassium carbonate (87.7 g; 0.634 mmol) in DMF (900 ml) was added methyl bromoacetate (30.00 ml; 0.317 mmol). The mixture was stirred vigorously overnight under nitrogen. The mixure was filtered and evaporated t dryness, *in vacuo*. The solid residue was treated with water (300 ml) and 4 N hydrochloric acid (12 ml), stirred for 15 min at 0°C, filtered, and washed with water (2 x 75 ml). The precipitate was treated with water (120 ml) and 2N sodium hydroxide (60 ml), and was boiled for 10 minutes. The mixture was cooled to 0°C, filtered, and the pure title compound was precipitated by the addition of 4 N hydrochloric acid (70 ml). Yield after drying, *in vacuo* over sicapent: 37.1 g (64%). ^1H-NMR: (90 MHz; DMSO-d_6): 11.33 ppm (s,1H,N<u>H</u>); 7.49(d,J=0.92Hz,1H,Ar<u>H</u>); 4.38 (s,2H,C<u>H</u>$_2$); 1.76 (d,J=0.92Hz,T-C<u>H</u>$_3$)

EXAMPLE 4

N-1-Carboxymethylthymine pentafluorophenyl ester (5)

N-1-Carboxymethylthymine (4, 10.0g; 54.3 mmol) and pentafluorophenol (10.0 g; 54.3 mmol) were dissolved in DMF (100 ml) and cooled to 5°C in ice water. DCC (13.45 g; 65.2 mmol) then was added. When the temperatur passed below 5°C, the ice bath was rem ved and the mixture was stirred for 3 h at ambient temperature. The precipitated DCU was rem ved by filtration and washed twice with DMF (2 x 10 ml). Th

combined filtrat was poured into ether (1400 ml) and cooled to 0°C. Petroleum ther (1400 ml) was added and the mixture was left overnight. The title compound was isolated by filtration and was washed thoroughly with petroleum ether. Yield: 14.8 g(78%). The product was pure enough to carry out the next reaction, but an analytical sample was obtained by recrystallization from 2-propanol. M.p. 200.5-206°C Anal. f r $C_{13}H_7F_5N_2O_4$. Found(calc.) C: 44.79(44.59); H: 2.14(2.01) N: 8.13(8.00). FAB-MS: 443 (M+1+glycerol), 351 (M+1). ^{1}H-NMR (90 MHz; DMSO-d_6): 11.52 ppm (s,1H,N<u>H</u>); 7.64 (s,1H,Ar<u>H</u>); 4.99 (s,2H,C<u>H</u>$_2$); 1.76 (s,3H,C<u>H</u>$_3$).

EXAMPLE 5

1-(Boc-aeg)thymine (6)

To the DMF-solution from above was added triethyl amine (7.08 ml; 50.8 mmol) followed by N-1-carboxymethylthymine pentafluorophenyl ester (5, 4.45 g; 12.7 mmol). The resultant solution was stirred for 1 h. The solution was cooled to 0°C and treated with cation exchange material ("Dowex 50W X-8", 40 g) for 20 min. The cation exchange material was removed by filtration, washed with dichloromethane (2 x 15 ml), and dichloromethane (150 ml) was added. The resulting solution was washed with saturated sodium chloride, dried over magnesium sulfate, and evaporated to dryness, *in vacuo*, first by a water aspirator and then by an oil pump. The residue was shaken with water (50 ml) and evaporated to dryness. This procedure was repeated once. The residue then was dissolved in methanol (75 ml) and poured into ether (600 ml) and petroleum ether (1.4 L). After stirring overnight, the white solid was isolated by filtration and was washed with petroleum ether. Drying over sicapent, *in vacuo*, gave 3.50 g (71.7%). M.p. 142-147°C. Anal. for $C_{16}H_{24}N_4O_7$. Found(calc.) C: 49.59(50.00) H: 6.34(6.29) N: 14.58(14.58). ^{1}H-NMR (250 MHz, DMSO-d_6): Due to the limited rotation around the secondary amide bond several of the signals were doubled in the ratio 2:1,(indicated in the list by mj. f r maj r and mi. for min r). 12.73 ppm (b,1H, -CO_2H); 11.27 ppm (s, mj., imide);

11.25 ppm (s, mi., imide); 7.30 ppm (s, mj., ArH); 7.26 ppm (s, mi., ArH); 6.92 ppm (unres. t, mj., BocNH); 6.73 ppm (unres. t; mi., BocNH); 4.64 ppm (s, mj., T-CH_2-CO-); 4.47 ppm (s, mi., T-CH_2-CO-); 4.19 ppm (s, mi., CONRC$\underline{H}_2CO_2$H); 3.97 ppm (s, mj., CONRC$\underline{H}_2CO_2\underline{H}$); 3.41-2.89 ppm (unres. m, -CH_2CH_2- and water); 1.75 ppm (s,3H, T-CH_3); 1.38 ppm (s, 9H, t-Bu). ^{13}C-NMR: 170.68 ppm (CO); 170.34 (CO); 167.47 (CO); 167.08 (CO); 164.29 (CO); 150.9 (C5''); 141.92 (C6''); 108.04 (C2'); 77.95 and 77.68 (Thy-C$\underline{H}_2$CO); 48.96, 47.45 and 46.70 (-$\underline{C}H_2\underline{C}H_2$- and NC$\underline{H}_2CO_2$H); 37.98 (Thy-C$\underline{H}_3$); 28.07 (t-Bu). FAB-MS: 407 (M+Na$^+$); 385 (M+H$^+$).

EXAMPLE 6

1-(Boc-aeg)thymine pentafluorophenyl ester (7, Boc-Taeg.OPfp)

1-(Boc-aeg)thymine (6) (2.00 g; 5.20 mmol) was dissolved in DMF (5 ml) and methylene chloride (15 ml) was added. Pentafluorophenol (1.05 g; 5.72 mmol) was added and the solution was cooled to 0°C in an ice bath. DDC then was added (1.29 g; 6.24 mmol) and the ice bath was removed after 2 min. After 3 h with stirring at ambient temperature, the precipitated DCU was removed by filtration and washed with methylene chloride. The combined filtrate was washed twice with aqueous sodium hydrogen carbonate and once with saturated sodium chloride, dried over magnesium sulfate, and evaporated to dryness, *in vacuo*. The solid residue was dissolved in dioxane (150 ml) and poured into water (200 ml) at 0°C. The title compound was isolated by filtration, washed with water, and dried over sicapent, *in vacuo*. Yield: 2.20 g (77%). An analytical sample was obtained by recrystallisation from 2-propanol. M.p. 174-175.5°C. Analysis for $C_{22}H_{23}N_4O_7F_5$, found(-calc.): C: 48.22(48.01); H: 4.64(4.21); N: 9.67(10.18). ^{1}H-NMR (250 MHz, $CDCl_3$):Due to the limited rotation around the secondary amide bond several of the signals were doubled in the rati 6:1 (indicated in the list by mj. for maj r and mi. for minor). 7.01 ppm (s, mi., ArH); 6.99 ppm (s, mj., ArH); 5.27 ppm (unres. t, B cN$\underline{H}$); 4.67 ppm (s, mj., T-CH_2-CO-); 4.60 ppm (s, mi., T-CH_2-CO-); 4.45 ppm (s, mj., CONRC$\underline{H}_2CO_2$Pfp); 4.42

ppm (s, mi., $CONRCH_2CO_2Pfp$); 3.64 ppm (t,2H,$BocNHCH_2CH_2$-); 3.87 ppm ("q",2H,$BocNHCH_2CH_2$-); 1.44(s,9H,t-Bu). FAB-MS: 551 (10; M+1); 495 (10; M+1-tBu); 451 (80; -Boc).

EXAMPLE 7

N^4-Benzyloxycarbonyl cytosine (9)

Over a period of about 1 h, benzyloxycarbonyl chloride (52 ml; 0.36 mol) was added dropwise to a suspension of cytosine (8, 20.0 g;0.18 mol) in dry pyridine (1000 ml) at 0°C under nitrogen in oven-dried equipment. The solution then was stirred overnight, after which the pyridine suspension was evaporated to dryness, *in vacuo*. Water (200 ml) and 4 N hydrochloric acid were added to reach pH ~1. The resulting white precipitate was filtered off, washed with water and partially dried by air suction. The still-wet precipitate was boiled with absolute ethanol (500 ml) for 10 min, cooled to 0°C, filtered, washed thoroughly with ether, and dried, *in vacuo*. Yield 24.7 g (54%). M.p.>250°C. Anal. for $C_{12}H_{11}N_3O_3$. Found(calc.); C: 58.59(58.77); H: 4.55(4.52); N: 17.17(17.13). No NMR spectra were recorded since it was not possible to get the product dissolved.

EXAMPLE 8

N^4-Benzyloxycarbonyl-N^1-carboxymethyl cytosine (10)

In a three necked round bottomed flask equipped with mechanical stirring and nitrogen coverage was placed methyl bromacetate (7.82 ml;82.6 mmol) and a suspension of N^4-benzyloxycarbonyl-cytosine (9, 21.0 g;82.6 mmol) and potassium carbonate (11.4 g;82.6 mmol) in dry DMF (900 ml). The mixture was stirred vigorously overnight, filtered, and evaporated to dryness, *in vacuo*. Water (300 ml) and 4 N hydrochloric acid (10 ml) were added, the mixture was stirred for 15 minutes at 0°C, filtered, and washed with water (2 x 75 ml). The isolated precipitate was treated with water (120 ml), 2N s dium hydr xide (60 ml), stirred for 30 min, filtered, cooled to 0°C, and 4 N hydrochloric acid (35 ml) was added. The title c mpound was is lated by filtration, washed

thoroughly with water, recrystallized from m thanol (1000 ml) and wash d thoroughly with ether. This afforded 7.70 g (31%) of pure compound. The mother liquor from the recrystallization was reduced to a volume of 200 ml and cooled to 0°C. This afforded an additional 2.30 g of a material that was pure by tlc but had a reddish color. M.p. 266-274°C. Anal. for $C_{14}H_{13}N_3O_5$. Found(calc.); C: 55.41(55.45); H: 4.23(4.32); N: 14.04(13.86). ^{1}H-NMR (90 MHz; DMSO-d_6): 8.02 ppm (d,J=7.32Hz,1H,H-6); 7.39 (s,5H,Ph); 7.01 (d,J=7.32Hz,1H,H-5); 5.19 (s,2H,PhC$\underline{H}_2$-); 4.52 (s,2H).

EXAMPLE 9

N^4-Benzyloxycarbonyl-N^1-carboxymethyl-cytosine pentafluorophenyl ester (11)

N^4-Benzyloxycarbonyl-N^1-carboxymethyl-cytosine (10, 4.00 g; 13.2 mmol) and pentafluorophenol (2.67 g; 14.5 mmol) were mixed with DMF (70 ml), cooled to 0°C with ice-water, and DCC (3.27 g; 15.8 mmol) was added. The ice bath was removed after 3 min and the mixture was stirred for 3 h at room temperature. The precipitated DCU was removed by filtration, washed with DMF, and the filtrate was evaporated to dryness, *in vacuo* (0.2 mmHg). The solid residue was treated with methylene chloride (250 ml), stirred vigorously for 15 min, filtered, washed twice with diluted sodium hydrogen carbonate and once with saturated sodium chloride, dried over magnesium sulfate, and evaporated to dryness, *in vacuo*. The solid residue was recrystallized from 2-propanol (150 ml) and the crystals were washed thoroughly with ether. Yield 3.40 g (55%). M.p. 241-245°C. Anal. for $C_{20}H_{12}N_3F_5O_5$. Found(calc.); C: 51.56(51.18); H: 2.77(2.58); N: 9.24(8.95). ^{1}H-NMR (90 MHz; $CDCl_3$): 7.66 ppm (d,J=7.63Hz,1H,H-6); 7.37 (s,5H,Ph); 7.31 (d,J=7.63Hz,1H,H-5); 5.21 (s,2H,PhC$\underline{H}_2$-); 4.97 (s,2H,NC$\underline{H}_2$-). FAB-MS: 470 (M+1)

EXAMPLE 10

N^4-Benzyl xycarb nyl-1-B c-aeg-cyt sine (12)

To a solution of (N-Boc-2-aminoethyl)glycine (2) in DMF, prepared as described above, was added triethyl amine (7.00 ml; 50.8 mmol) and N^4-benzyloxycarbonyl-N^1-carboxymethyl-cytosine pentafluorophenyl ester (11, 2.70 g; 5.75 mmol). After stirring the solution for 1 h at room temperature, methylene chloride (150 ml), saturated sodium chloride (250 ml), and 4 N hydrochloric acid to pH ~1 were added. The organic layer was separated and washed twice with saturated sodium chloride, dried over magnesium sulfate, and evaporated to dryness, *in vacuo*, first with a water aspirat r and then with an oil pump. The oily residue was treated with water (25 ml) and was again evaporated to dryness, *in vacuo*. This procedure then was repeated. The oily residue (2.80 g) was then dissolved in methylene chloride (100 ml), petroleum ether (250 ml) was added, and the mixture was stirr d overnight. The title compound was isolated by filtration and washed with petroleum ether. Tlc (system 1) indicated substantial quantities of pentafluorophenol, but no attempt was made to remove it. Yield: 1.72 g (59%). M.p. 156°C(decomp.). ^{1}H-NMR (250 MHz, $CDCl_3$): Due to the limit d rotation around the secondary amide bond several of the signals were doubled in the ratio 2:1,(indicated in the list by mj. for major and mi. for minor). 7.88 ppm (dd,1H,H-6); 7.39 (m,5H,Ph); 7.00 (dd,1H,H-5); 6.92 (b,1H,BocN$\underline{H}$); 6.74 (b,1H,ZN$\underline{H}$)-?; 5.19 (s,2H,Ph-C$\underline{H}_3$); 4.81 ppm (s, mj., Cyt-CH_2-CO-); 4.62 ppm (s, mi., Cyt-CH_2-CO-); 4.23 (s, mi., CONRC$\underline{H}_2$$CO_2$H); 3.98 ppm (s, mj., CONRC$\underline{H}_2$$CO_2$H); 3.42-3.02 (unres. m, -CH_2CH_2- and water);1.37 (s,9H,tBu). FAB-MS: 504 (M+1); 448 (M+1-tBu).

EXAMPLE 11

N^4-Benzyloxycarbonyl-1-Boc-aeg-cytosine pentafluorophenyl ester (13)

N^4-Benzyl xycarbonyl-1-B c-aeg-cytosine (12, 1.50 g; 2.98 mmol) and pentaflu rophenol (548 mg; 2.98 mmol) was

dissolved in DMF (10 ml) M thylene chloride (10 ml) was added, the reaction mixtur was cooled t 0°C in an ice bath, and DCC (676 mg; 3.28 mmol) was added. The ice bath was removed after 3 min and the mixture was stirred for 3 h at ambient temperature. The precipitate was isolated by filtration and washed once with methylene chloride. The precipitate was dissolved in boiling dioxane (150 ml) and the solution was cooled to 15°C, whereby DCU precipitated. The DCU was removed by filtration and the resulting filtrate was poured into water (250 ml) at 0°C. The title compound was isolated by filtration, was washed with water, and dried over sicapent, *in vacuo*. Yield 1.30 g (65%). Analysis for $C_{29}H_{28}N_5O_8F_5$. Found(calc.); C: 52.63(52.02); H: 4.41(4.22); N: 10.55(10.46). ^{1}H-NMR (250 MHz; DMSO-d_6): showed essentially the spectrum of the above acid, most probably due to hydrolysis of the ester. FAB-MS: 670 (M+1); 614 (M+1-tBu)

EXAMPLE 12

4-Chlorocarboxy-9-chloroacridine

4-Carboxyacridone (6.25 g; 26.1 mmol), thionyl chloride (25 ml), and 4 drops af DMF were heated gently under a flow of nitrogen until all solid material had dissolved. The solution then was refluxed for 40 min. The solution was cooled and excess thionyl chloride was removed *in vacuo*. The last traces of thionyl chloride were removed by coevaporation with dry benzene (dried over Na-Pb) twice. The remaining yellow powder was used directly in the next reaction.

EXAMPLE 13

4-(5-Methoxycarbonylpentylamidocarbonyl)-9-chloroacridine

Methyl 6-aminohexanoate hydrochloride (4.70 g; 25.9 mmol) was dissolved in methylene chloride (90 ml), cooled to 0°C, triethyl amine (15 ml) was added, and the resulting solution then was immediat ly added to the acid chloride from above. The roundbottomed flask c ntainingthe acid chloride was c oled to 0°C in an ic bath. Th mixture was stirr d vigor usly for 30 min at 0°C and 3 h at r om temperature. The

resulting mixture was filtered to rem ve the remaining solids, which were wash d with methylene chloride (20 ml). The red-brown methylene chloride filtrate was subsequently washed twice with saturated sodium hydrogen carbonate, once with saturated sodium chloride, dried over magnesium sulfate, and evaporated to dryness, *in vacuo*. To the resulting oily substance was added dry benzene (35 ml) and ligroin (60-80°C, dried over Na-Pb). The mixture was heated to reflux. Activated carbon and celite were added and mixture was refluxed for 3 min. After filtration, the title compound crystallised upon cooling with magnetic stirring. It was isolated by filtration and washed with petroleum ether. The product was stored over solid potassium hydroxide. Yield 5.0 g (50%).

EXAMPLE 14

4-(5-Methoxycarbonylpentyl)amidocarbonyl-9-[6'-(4''-nitrobenzamido)hexylamino]-aminoacridine

4-(5-Methoxycarbonylpentylamidocarbonyl)-9-chloroacridine (1.30 g; 3.38 mmol) and phenol (5 g) were heated to 80°C for 30 min under a flow of nitrogen, after which 6-(4'-nitrobenzamido)-1-hexylamine (897 mg; 3.38 mmol) was added. The temperature raised to 120°C for 2 h. The reaction mixture was cooled and methylene chloride (80 ml) was added. The resulting solution was washed three times with 2N sodium hydroxide (60 ml portions) and once with water, dried over magnesium sulfate, and evaporated to dryness, *in vacuo*. The resulting red oil (1.8 g) was dissolved in methylene chloride (40 ml), cooled to 0°C. Ether (120 ml) was added and the resultant solution was stirred overnight. This results in a mixture of solid material and an oil. The solid was isolated by filtration. The solid and the oil were re-dissolved in methylene chloride (80 ml) and added dropwise to cold ether (150 ml). After 20 minutes of stirring, the title compound was isolated by filtration in the f rm of range crystals. The pr duct was washed with ether and dried *in*

vacuo over potassium hydroxide. Yield 1.60 g (77%). M.p. 145-147°C.

EXAMPLE 15

4-(5-Carboxypentyl)amidocarbonyl-9-[6'-(4''-nitrobenzamido)hexylamino]-aminoacridine

4-(5-Methoxycarbonylpentyl)amidocarbonyl-9-[6'-(4''-nitrobenzamido)hexylamino]aminoacridine (503 mg; 0.82 mmol) was dissolved in DMF (30 ml), and 2 N sodium hydroxide (30 ml) was added. After stirring for 15 min, 2 N hydrochloric acid (35 ml) and water (50 ml) were added at 0°C. After stirring for 30 min, the solution was decanted, leaving an oily substance which was dissolved in boiling methanol (150 ml), filtered and concentrated to 1/3 volume. To the methanol solution were added ether (125 ml) and 5-6 drops of HCl in ethanol. The solution was decanted after 1 h of stirring at 0°C. The oily substance was redissolved in methanol (25 ml) and precipitated with ether (150 ml). The title compound was isolated as yellow crystals after stirring overnight. Yield 417 mg (80%). M.p. 173°C (decomp.).

EXAMPLE 16

(a) 4-(5-pentafluorophenyloxycarbonylpentyl)-amidocarbonyl-9-[6'-(4''-nitrobenzamido)-hexylamino]-aminoacridine(Acr^{1}Opfp)

The acid from above (300 mg; 0.480 mmol) was dissolved in DMF (2 ml) and methylene chloride (8 ml) was added. Pentafluorophenol (97 mg; 0.53 mmol), transferred with 2 x 2 ml of the methylene chloride, was added. The resulting solution was cooled to 0°C after which DCC (124 mg; 0.60 mmol) was subsequently added. The ice bath was removed after 5 minutes and the mixture was left with stirring overnight. The precipitated DCU was removed by centrifugation and the centrifugate was evaporated to dryness, *in vacuo*, first by a water aspirator and then by an oil pump. The residue was diss lved in methylen chl ride (20 ml), filtered, and evap - rated to dryness, *in vacuo*. The r sidue was again dissolved

in methyl ne chloride and petroleum ether (150 ml). A 1 ml portion of 5M HCl in ether was added. The solvent was removed by decanting after 30 min of stirring at 0°C. The residual oily substance was dissolved in methylene chloride (100 ml). Petroleum ether (150 ml) was added and the mixture was left with stirring overnight. The next day the yellow precipitat d crystalline material was isolated by filtration and was washed with copious amounts of petroleum ether. Yield, after drying, 300 mg (78%). M.p. 97.5°C (decomp.) All samples showed satisfactory elemental analysis, ^{1}H- and ^{13}C-NMR and mass spectra.

(b) Experimental for the synthesis of PNA compounds, cf. Figure 8

Materials: Boc-Lys (ClZ), benzhydrylamine-copoly-(styrene-1%-divinylbenzene) resin (BHA resin), and p-methylbenzhydrylamine-copoly(styrene-1%-divinylbenzene)resin (MBHA resin) were purchased from Peninsula Laboratories. Other reagents and solvents were: Biograde trifluoroacetic acid from Halocarbon Products; diisopropylethylamine (99%; was not further distilled) and N-acetylimidazole (98%) from Aldrich; H_2O was distilled twice; anhydrous HF from Union Carbide; synthesis grade N,N-dimethylformamide and analytical grade methylene chloride (was not further distilled) from Merck; HPLC grade acetonitrile from Lab-Scan; purum grade anisole, N,N'-dicyclohexylcarbodiimide, diisopropylcarbodiimide, puriss. grade 2,2,2-trifluoroethanol from Fluka and trifluoromethanesulfonic acid from flourad.

(b) General Methods and Remarks

Except where otherwise stated, the following applies. The PNA compounds were synthezised by the stepwise solid-phas approach (Merrifield, *J. Am. Chem. Soc.*, 1963, *85*, 2149) employing conventional peptide chemistry utilizing the TFA-labile *tert*-butyloxyoarbonyl (Boc) group for "temporary" N-protection (Merrifield, *J. Am. Chem. Soc.*, 1964, *86*, 304) and the more acid-stable b nzyloxycarb nyl (Z) and 2-chl r benzyloxycarb nyl (ClZ) groups for "permanent" side chain prot ction. To obtain C-terminal amides, the PNAs w re assembl d onto the HF-labil BHA or MBHA resins (the MBHA

resin has increased susceptibility to the final HF cleavage relative to th unsubstitut d BHA resin (Matsueda, *et al.*, *Peptides*, 1981, *2*, 45). All reactions (except HF reactions) were carried out in manually operated standard solid-phase reaction vessels fitted with a coarse glass frit (Merrifield, *et al.*, *Biochemistry*, 1982, *21*, 5020). The quantitative ninhydrin reaction (Kaiser test), originally developed by Merrifield and co-workers (Sarin, *et al.*, *Anal. Biochem.*, 1981, *117*, 147) for peptides containing "normal" amino acids, was successfully appplied (see Table I - III) using the "normally" employed effective extinction coefficient $\epsilon = 15000$ $M^{-1}cm^{-1}$ for all residues to determine the completeness of the individual couplings as well as to measure the number of growing peptide chains. The theoretical substitution S_{n-1} upon coupling of residue number n (assuming both complete deprotection and coupling as well as neither chain termination nor loss of PNA chains during the synthetic cycle) is calculated from the equation:

$$S_n = S_{n-1} \times (1 + (S_{n-1} \times \Delta MW \times 10^{-3} \text{ mmol/mol}))^{-1}$$

where ΔMW is the gain in molecular weight ($[\Delta MW]$ = g/mol) and S_{n-1} is the theoretical substitution upon coupling of the preceding residue n-1 ([S] = mmol/g). The estimated value (%) on the extent of an individual coupling is calculated relative to the measured substitution (unless S was not determined) and include correction for the number of remaining free amino groups following the previous cycle. HF reactions were carried out in a Diaflon HF apparatus from Toho Kasei (Osaka, Japan). Vydac C_{18} (5 μm, 0.46 x 25 cm and 5 μm, 1.0 x 25 cm) reverse-phase columns, respectively were used for analytical and semi-preparative HPLC on an SP8000 instrument. Buffer A was 5 vol % acetonitrile in water containing 445 μl trifluoroacetic acid per liter, and buffer B was 60 vol % acetonitrile in water containing 390 μl trifluoroacetic acid per liter. The linear gradient was 0-100% of buffer B in 30 min, flow rates 1.2 ml/min (analytical) and 5 ml/min (semi-preparative). The eluents were m nit red at 215 nm (analytical) and 230 nm (semi-preparative). M lecular weights

f the PNAs were determined by ^{252}Cf plasma desorption time-of-flight mass spectrometry from th mean of the m st abundant isotopes.

EXAMPLE 17

Solid-Phase Synthesis of Acr^{1}-$[Taeg]_{15}$-NH_2 and Short r Derivatives

(a) Stepwise Assembly of Boc-$[Taeg]_{15}$-BHA Resin

The synthesis was initiated on 100 mg of preswollen and neutralized BHA resin (determined by the quantitative ninhydrin reaction to contain 0.57 mmol NH_2/g) employing single couplings ("Synthetic Protocol 1") using 3.2 equivalents of BocTaeg-OPfp in about 33% DMF/CH_2Cl_2. The individual coupling reactions were carried out by shaking for at least 12 h in a manually operated 6 ml standard solid-phase reaction vessel and unreacted amino groups were blocked by acetylation at selected stages of the synthesis. The progress of chain elongation was monitored at several stages by the quantitative ninhydrin reaction (see Table I). Portions of protected Boc-$[Taeg]_5$-BHA, Boc-$[Taeg]_{10}$-BHA, and Boc-$[Taeg]_{15}$-BHA resins were taken out after assembling 5, 10, and 15 residues, respectively.

Synthetic Step	Residue Coupled	Substitution After Deprotection (mmol/g)		Remaining Free Amino Groups After (μmol/g)		Estimated Extent of Coupling
		Measd	Theoretcl	Single Coupling	Acetylation	(%)
"0"		0.57				
1	BocTaeg	ND	0.50	1.30		<99.7
2	BocTaeg	ND	0.44	1.43		<99.9
3	BocTaeg	0.29	0.39	3.33		99.3
4	BocTaeg	0.27	0.35	13.30		96.3
5	BocTaeg	0.26	0.32	8.33		>99.9
6	BocTaeg	ND	0.30	7.78		>99.9
7	BocaTeg	ND	0.28	13.81	7.22	<97.8
8	BocTaeg	ND	0.26	14.00		<99.9
9	BocTaeg	ND	0.24	30.33		93.2
10	BocTaeg	0.16	0.23	11.67	2.67	>99.9
11	BocTaeg	ND	0.21	4.58		>99.9
12	BocTaeg	ND	0.20	5.87		<99.4
13	BocTaeg	ND	0.19	1.67		>99.9
14	BocTaeg	ND	0.18	14.02		<93.1
15	BocTaeg	0.07	0.17	4.20	3.33	>99.9

(b) Synthesis of Acr[1]-$[Taeg]_{15}$-BHA Resin

Following deprotection of the residual Boc-$[Taeg]_{15}$-BHA resin (estimated dry weight is about 30 mg; ~0.002 mmol growing chains), the H-$[Taeg]_{15}$-BHA resin was reacted with about 50 equivalents (80 mg; 0.11 mmol) of Acr[1]-OPfp in 1 ml of about 66% DMF/CH_2Cl_2 (*i.e.*, a 0.11 M solution of the pentafluorophenylester) in a 3 ml solid-phase reaction vessel. As judged by a qualitative ninhydrin reaction, coupling of the acridine moiety was close to quantitative.

(c) Cleavage, Purification, and Identification of H-$[Taeg]_5$-NH_2

A portion of protected Boc-$[Ta g]_5$-BHA resin was treated with 50% trifluor acetic acid in methylene chl ride to remove the N-terminal Boc gr up (which is a precursor of

the potentially harmful *tert*-butyl cati n) pri r t th HF cleavage. Following neutralization and washing (performed in a way similar to those of steps 2-4 in "Synthetic Protoc l 1"), and drying for 2 h in vacuum, the resulting 67.1 mg (dry weight) of H-[Taeg]$_5$-BHA resin was cleaved with 5 ml f HF:anisole (9:1, v/v) stirring at 0°C for 60 min. . After removal of HF, the residue was stirred with dry diethyl ether (4 x 15 ml, 15 min each) to remove anisole, filtered under gravity through a fritted glass funnel, and dried. The PNA was then extracted into a 60 ml (4 x 15 ml, stirring 15 min each) 10% aqueous acetic acid solution. Aliquots of this solution were analyzed by analytical reverse-phase HPLC to establish the purity of the crude PNA. The main peak at 13.0 min accounted for about 93% of the total absorbance. The remaining solution was frozen and lyophilized to afford about 22.9 mg of crude material. Finally, 19.0 mg of the crude product was purified from five batches, each containing 3.8 mg in 1 ml of H_2O. The main peak was collected by use of a semi-preparative reverse-phase column. Acetonitrile was removed on a speed vac and the residual solution was frozen (dry ice) and subsequently lyophilized to give 13.1 mg of >99% pure H-[Taeg]$_5$-NH_2. The PNA molecule readily dissolved in water and had the correct molecular weight based on mass spectral determination. For $(M+H)^+$ the calculated m/z value was 1349.3 and the measured m/z value was 1347.8.

(d) Cleavage, Purification, and Identification of H-[Taeg]$_{10}$-NH_2

A portion of protected Boc-[Taeg]$_{10}$-BHA resin was treated as described in section (c) to yield 11.0 mg of crude material upon HF cleavage of 18.9 mg dry H-[Taeg]$_{10}$-BHA resin. The main peak at 15.5 min accounted for about 53% of the total absorbance. About 1 mg of the crude product was purified repeatedly (for reasons described below) to give approximately 0.1 mg of at least 80% but presumably >99% pure H-[Taeg]$_{10}$-NH_2. A rather broad tail eluting after the target peak and accounting f r about 20% of the t tal abs rbance c uld not be removed (only slightly reduced) upon th r peated

purification. Judged by th mass spectrum, which only c nfirms the presence of the correct molecular weight H-$[Taeg]_{10}$-NH_2, the tail phenomonen is ascribed to more or less well-defined aggregational/conformational states of the target molecule. Therefore, the crude product is likely to contain more than the above-mentioned 53% of the target molecule. H-$[Taeg]_{10}$-NH_2 is readily dissolved in water. For $(M+H)^+$ the calculated m/z value was 2679.6 and the measured m/z value was 2681.5.

(e) Cleavage, Purification, and Identification of H-$[Taeg]_{15}$-NH_2.

A portion of protected Boc-$[Taeg]_{15}$-BHA resin was treated as described in section (c) to yield 3.2 mg of crude material upon HF cleavage of 13.9 mg dry H-$[Taeg]_{15}$-BHA resin. The main peak at 22.6 min was located in a broad bulge accounting for about 60% of the total absorbance (Fig. 12a). Again (see the preceding section), this bulge is ascribed to aggregational/conformational states of the target molecule H-$[Taeg]_{15}$-NH2 since mass spectral analysis of the collected "bulge" did not significantly reveal the presence of other molecules. All of the crude product was purified collecting the "bulge" to give approximately 2.8 mg material. For $(M+Na)^+$ the calculated m/z value was 4033.9 and the measured m/z value was 4032.9.

(f) Cleavage, Purification, and Identification of Acr^1-$[Taeg]_{15}$-NH_2.

A portion of protected Acr^1-$[Taeg]_{15}$-BHA resin was treated as described in section (b) to yield 14.3 mg of crude material upon HF cleavage of 29.7 mg dry Acr^1-$[Taeg]_{15}$-BHA resin. Taken together, the main peak at 23.7 min and a "dimer" (see below) at 29.2 min accounted for about 40% of the total absorbance (Fig. 12b). The crude product was purified repeatedly to give approximately 1 mg of presumably >99% pure Acr^1-$[Ta g]_{15}$-NH_2 "c ntaminated" with self-aggregat d m lecules eluting at 27.4 min, 29.2 min, and finally as a huge broad bulge eluting with 100% buffer B (Fig. 12c). This interpretation is in agreem nt with the observation that thos

p aks grow upon standing (f r hours) in aqueous acetic acid solution, and finally precipitate out quantitatively. For $(M+H)^+$ the calculated m/z value was 4593.6 and the measured m/z value was 4588.7.

(g) Synthetic Protocol 1

(1) Boc-deprotection with TFA/CH_2Cl_2 (1:1, v/v), 3 ml, 3 x 1 min and 1 x 30 min; (2) washing with CH_2Cl_2, 3 ml, 6 x 1 min; (3) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 3 ml, 3 x 2 min; (4) washing with CH_2Cl_2, 3 ml, 6 x 1 min, and drain for 1 min; (5) 2-5 mg sample of PNA-resin may be taken out and dried thoroughly for a quantitative ninhydrin analysis to determine the substitution; (6) addition of 3.2 equiv. (0.18 mmol; 100 mg) BocTaeg-OPfp dissolved in 1 ml CH_2Cl_2 follow d by addition of 0.5 ml DMF (final concentration of pentafluorophenylester ~0.12 M); the coupling reaction was allowed to proceed for a total of 12-24 h shaking at ro m temperature; (7) washing with DMF, 3 ml, 1 x 2 min; (8) washing with CH_2Cl_2, 3 ml, 4 x 1 min; (9) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 3 ml, 2 x 2 min; (10) washing with CH_2Cl_2, 3 ml, 6 x 1 min; (11) 2-5 mg sample of protected PNA-resin is taken out for a rapid qualitative ninhydrin test and further 2-5 mg is dried thoroughly for a quantitative ninhydrin analysis to determine the extent of coupling (after cycles 7, 10, and 15 unreacted amino groups were blocked by acetylation with N-acetylimidazol in methylene chloride).

EXAMPLE 18

Solid-Phase Synthesis of Acr^1-$[Taeg]_{15}$-Lys-NH_2 and Shorter Derivatives

(a) Stepwise Assembly of Boc-$[Taeg]_{15}$-Lys(ClZ)-BHA Resin

The synthesis was initiated by a quantitative loading (standard DCC *in situ* coupling in neat CH_2Cl_2) of Boc-Lys(ClZ) onto 100 mg of preswollen and neutralized BHA resin (0.57 mmol NH_2/g). Further extension of the protected PNA chain employed single c uplings ("Synthetic Pr tocol 2") f r cycles 1 to 5 and cycles 10 to 15 using 3.2 equivalents f BocTaeg-

OPfp in ab ut 33% DMF/CH_2Cl_2. Cycles 5 to 10 employed an extra straight DCC (*i. ., in situ*) c upling of the free acid BocTaeg-OH in about 33% DMF/CH_2Cl_2. All coupling reactions were carried out by shaking for at least 12 h in a manually operated 6 ml standard solid-phase reaction vessel. Unreacted amino groups were blocked by acetylation at the same. stages of the synthesis, as was done in Example 17. Portions of protected Boc-$[Taeg]_5$-Lys(ClZ)-BHA and Boc-$[Taeg]_{10}$-Lys(ClZ)-BHA resins were taken out after assembling 5 and 10 PNA residues, respectively. As judged by the analytical HPLC chromatogram of the crude cleavage product from the Boc-$[Taeg]_{10}$-Lys(ClZ)-BHA resin (see section (e)), an additional "free acid" coupling of PNA residues 5 to 10 gave no significant improvement of the synthetic yield as compared to the throughout single-coupled residues in Example 17.

(b) Synthesis of Acr^1-$[Taeg]_{10}$-Lys(ClZ)-BHA Resin

Following deprotection of a portion of Boc-$[Taeg]_{10}$-Lys(ClZ)-BHA resin (estimated dry weight is about 90 mg; ~ 0.01 mmol growing chains), the H-$[Taeg]_{15}$-BHA resin was reacted with about 20 equivalents (141 mg; 0.19 mmol) of Acr^1-OPfp in 1 ml of about 66% DMF/CH_2Cl_2 in a 3 ml solid-phase reaction vessel. As judged by a qualitative ninhydrin reaction, coupling of the acridine moiety was close to quantitative.

(c) Synthesis of Acr^1-$[Taeg]_{15}$-Lys(ClZ)-BHA Resin

Following deprotection of the residual Boc-$[Taeg]_{15}$-Lys(ClZ)-BHA resin (estimated dry weight about 70 mg; ~ 0.005 mmol growing chains), the H-$[Taeg]_{15}$-Lys(ClZ)-BHA resin was reacted with about 25 equivalents (91 mg; 0.12 mmol) of Acr^1-OPfp in 1 ml of about 66% DMF/CH_2Cl_2 in a 3 ml solid-phase reaction vessel. As judged by a qualitative ninhydrin reaction, coupling of the acridine moiety was close to quantitative.

(d) Cleavage, Purificati n, and Id ntificati n of H-$[Ta g]_5$-Lys-NH_2

A portion of protected B c-$[Taeg]_5$-Lys(ClZ)-BHA resin was treated as described in Example 17c to yield 8.9 mg of

crude material upon HF cleavage f 19.0 mg dry H-[Ta g]$_5$-Lys(ClZ)-BHA resin. The main p ak at 12.2 min (eluted at 14.2 min if injected from an aqueous solution instead of the 10% aqueous acetic acid solution) accounted for about 90% of the total absorbance. About 2.2 mg of the crude product was purified to give approximately 1.5 mg of 99% pure H-[Taeg]$_5$-Lys-NH_2.

(e) **Cleavage, Purification, and Identification of H-[Taeg]$_{10}$-Lys-NH_2**

A portion of protected Boc-[Taeg]$_{10}$-Lys(ClZ)-BHA resin was treated as described in Example 17c to yield 1.7 mg of crude material upon HF cleavage of 7.0 mg dry H-[Taeg]$_{10}$-Lys(ClZ)-BHA resin. The main peak at 15.1 min (eluted at 17.0 min if injected from an aqueous solution instead of the 10% aqueous acetic acid solution) accounted for about 50% of the total absorbance. About 1.2 mg of the crude product was purified to give approximately 0.2 mg of >95% pure H-[Taeg]$_{10}$-Lys-NH_2. Figure 13a. For $(M+H)^+$ the calculated m/z value was 2807.8 and the measured m/z value was 2808.2.

(f) **Cleavage, Purification, and Identification f Acr^1-[Taeg]$_{10}$-Lys-NH_2**

99.1 mg protected Acr^1-[Taeg]$_{10}$-Lys(ClZ)-BHA resin (dry weight) was cleaved as described in Example 17c to yield 42.2 mg of crude material. The main peak at 25.3 min (eluted at 23.5 min if injected from an aqueous solution instead of the 10% aqueous acetic acid solution) accounted for about 45% of the total absorbance. An 8.87 mg portion of the crude product was purified to give approximately 5.3 mg of >97% pure H-[Taeg]$_{10}$-Lys-NH_2. For $(M+H)^+$ the calculated m/z value was 2850.8 and the measured m/z value was 2849.8.

(g) **Cleavage and Purification of Acr^1-[Taeg]$_{15}$-Lys-NH_2**

A 78.7 mg portion of protected Acr^1-[Taeg]$_{15}$-Lys(ClZ)-BHA resin (dry weight) was cleaved as described in Example I section (c) to yield 34.8 mg of crude material. The main peak at 23.5 min (about the same eluti n time if injected from an aqueous solution instead of the 10% aque us ac tic acid solution) and a "dimer" at 28.2 min acc unted for ab ut 35%

f the total absorbanc . About 4.5 mg of the crude product was purified to give approximat ly 1.6 mg of presumably >95% pure H-$[Taeg]_{10}$-Lys-NH_2. This compound could not be free of the "dimer" peak, which grew upon standing in aqueous acetic acid solution.

(h) Synthetic Protocol 2

(1) Boc-deprotection with TFA/CH_2Cl_2 (1:1, v/v), 3 ml, 3 x 1 min and 1 x 30 min; (2) washing with CH_2Cl_2, 3 ml, 6 x 1 min; (3) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 3 ml, 3 x 2 min; (4) washing with CH_2Cl_2, 3 ml, 6 x 1 min, and drain for 1 min; (5) 2-5 mg sample of PNA-resin can be taken out and dried thoroughly for a qualitative ninhydrin analysis; (6) for cycles 1 to 5 and cycles 10 to 15 the coupling reaction was carried out by addition of 3.2 equiv. (0.18 mmol; 100 mg) BocTaeg-OPfp dissolved in 1 ml CH_2Cl_2 followed by addition of 0.5 ml DMF (final concentration of pentafluorophenylester ~ 0.12 M); the coupling reaction was allowed to proceed for a total of 12-24 h with shaking; cycles 5 to 10 employed an additional 0.12 M DCC coupling of 0.12 M BocTaeg-OH in 1.5 ml DMF/CH_2Cl_2 (1:2, v/v); (7) washing with DMF, 3 ml, 1 x 2 min; (8) washing with CH_2Cl_2, 3 ml, 4 x 1 min; (9) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 3 ml, 2 x 2 min; (10) washing with CH_2Cl_2, 3 ml, 6 x 1 min; (11) 2-5 mg sample of protected PNA-resin is taken out for a qualitative ninhydrin test (after cycles 7, 10, and 15 unreacted amino groups were blocked by acetylation with N-acetylimidazol in methylene chloride).

EXAMPLE 19

Improved Solid-Phase Synthesis of H-$[Taeg]_{10}$-Lys-NH_2

The protected PNA was assembled onto an MBHA resin, using approximately half the loading of the BHA resin used in the previous examples. Furthermore, all cycles except one was followed by acetylation of uncoupled amino groups. The following describes the synthesis in full detail:

(a) Preparati n of Boc-Lys(ClZ)-NH-CH(p-CH_3-C_6H_4)-C_6H_4 R sin (MBHA Resin) with an Initial Substitution f 0.3 mmol/g

The desired substitution of Boc-Lys(ClZ)-MBHA resin was 0.25 - 0.30 mmol/g. In order to get this value, 1.5 mmol of Boc-Lys(ClZ) was coupled to 5.0 g of neutralized and preswollen MBHA resin (determined by the quantitative ninhydrin reaction to contain 0.64 mmol NH_2/g) using a single "in situ" coupling (1.5 mmol of DCC) in 60 ml of CH_2Cl_2. The reaction was carried out by shaking for 3 h in a manually operated, 225 ml, standard, solid-phase reaction vessel. Unreacted amino groups were then blocked by acetylation with a mixture of acetic anhydride/pyridine/CH_2Cl_2 (1:1:2, v/v/v) for 18 h. A quantitative ninhydrin reaction on the neutralized resin showed that only 0.00093 mmol/g free amine remained (see Table I), *i.e.* 0.15% of the original amin groups. The degree of substitution was estimated by deprotection and ninhydrin analysis, and was found to be 0.32 mmol/g for the neutralized H-Lys(ClZ)-MBHA resin. This compares well with the maximum value of 0.28 mmol/g for a quantitative coupling of 0.30 mmol Boc-Lys(ClZ)/g resin (see Table II).

(b) Stepwise Assembly of Boc-$[Taeg]_3$-Lys(ClZ)-MBHA Resin

The entire batch of H-Lys(ClZ)-MBHA resin prepared in section (a) was used directly (in the same reaction vessel) to assemble Boc-$[Taeg]_3$-Lys(ClZ)-MBHA resin by single couplings ("Synthetic Protocol 3") utilizing 2.5 equivalents of BocTaeg-OPfp in neat CH_2Cl_2. The quantitative ninhydrin reaction was appplied throughout the synthesis (see Table II).

(c) Stepwise Assembly of Boc-$[Taeg]_8$-Lys(ClZ)-MBHA Resin

About 4.5 g of wet Boc-$[Taeg]_3$-Lys(ClZ)-MBHA resin (~0.36 mmol growing chains; taken out of totally ~ 19 g wet resin prepared in section (b)) was plac d in a 55 ml SPPS reaction vessel. B c-$[Taeg]_8$-Lys(ClZ)-MBHA resin was assembled by single couplings ("Synthetic Protocol 4")

utilizing 2.5 equivalents of B cTaeg-OPfp in about 30% DMF/CH_2Cl_2. The progress of the synthesis was monitored at all stages by the quantitative ninhydrin reaction (see Table II).

(d) Stepwise Assembly of Boc-[Taeg]$_{10}$-Lys(ClZ)-MBHA Resin

About 1 g of wet Boc-[Taeg]$_8$-Lys(ClZ)-MBHA resin (~0.09 mmol growing chains; taken out of totally ~4 g wet resin prepared in section (c)) was placed in a 20 ml SPPS reaction vessel. Boc-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin was assembled by the single-coupling protocol employed in the preceding section utilizing 2.5 equivalents of BocTaeg-OPfp in about 30% DMF/CH_2Cl_2. The reaction volume was 3 ml (vigorous shaking). The synthesis was monitored by the quantitative ninhydrin reaction (see Table II).

Synthetic Step	Residue Coupled	Substitution After Deprotection (mmol/g)		Remaining Free Amino Groups After (μmol/g)		Estimated Extent of Coupling
		Measd	Theoret	Single Coupling	Acetylation	(%)
"0"	BocLys(ClZ)	0.32	0.28		0.93	
1	BocTaeg	0.23	0.26	0.97	0.54	>99.9
2	BocTaeg	0.21	0.24	0.92	0.46	99.8
3	BocTaeg	0.19	0.23	1.00	0.57	99.7
4	BocTaeg	0.18	0.21	1.85		99.3
5	BocTaeg	0.17	0.20	2.01	0.19	99.9
6	BocTaeg	0.15	0.19	1.69	0.10	99.0
7	BocaTeg	0.11	0.18	1.11	0.66	99.1
8	BocTaeg	0.12	0.17	1.82	0.44	99.0
9	BocTaeg	0.10	0.17	5.63	0.56	94.8
10	BocTaeg	0.11	0.16	1.54	0.67	99.1

(e) Synthesis of Ac-[Ta g]$_{10}$-Lys(ClZ)-MBHA Resin

Foll wing deprotecti n of a p rti n of Boc-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin (estimated dry weight is about 45 mg), the resin was next acetylated quantitatively with a 2 ml mixture

f acetic anhydride/pyridin /CH_2Cl_2 (1:1:2, v/v/v) for 2 h in a 3 ml s lid-phase reaction vessel.

(f) **Cleavage, Purification, and Identification of H-[Taeg]$_{10}$-Lys-NH_2**

A portion of protected Boc-[Taeg]$_{10}$-Lys(ClZ)-BHA resin was treated as described in Example 17c to yield about 24 mg of crude material upon HF cleavage of 76 mg dry H-[Taeg]$_5$-Lys(ClZ)-BHA resin. The main peak at 15.2 min (which includes impurities such as deletion peptides and various byproducts) accounted for about 78% of the total absorbance. The main peak also accounted for about 88% of the "main peak plus deletion peaks" absorbance, which is in good agreement with the overall estimated coupling yield of 90.1% obtained by summarizing the individual coupling yields in Table II. A 7.2 mg portion of the crude product was purified from two batches by use of a semi-preparative reserse-phase column, (collecting the main peak in a beaker cooled with dry ice/2-propanol). Each contained 3.6 mg in 1 ml of H_2O. The frozen solution was lyophilized directly (without prior removal of acetonitrile on a speed vac) to give 4.2 mg of 82% pure H-[Taeg]$_{10}$-Lys-NH_2.

(g) **Cleavage, Purification, and Identification of Ac-[Taeg]$_{10}$-Lys-NH_2**

A 400.0 mg portion of protected Ac-[Taeg]$_{10}$-Lys(ClZ)-BHA resin (dry weight) was cleaved as described in Exampl 17c, except for the TFA treatment to yield 11.9 mg of crude material. The main peak at 15.8 min accounted for about 75% of the total absorbance. A 4.8 mg portion of the crude product was purified to give approximately 3.5 mg of >95% pure Ac-[Taeg]$_{10}$-Lys-NH_2. For $(M+H)^+$ the calculated m/z value = 2849.8 and the measured m/z value = 2848.8.

(h) **Synthetic Protocol 3.**

(1) Boc-deprotection with TFA/CH_2Cl_2 (1:1, v/v), 100 ml, 3 x 1 min and 1 x 30 min; (2) washing with CH_2Cl_2, 100 ml, 6 x 1 min; (3) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 100 ml, 3 x 2 min; (4) washing with CH_2Cl_2, 100ml, 6 x 1 min, and drain for 1 min; (5) 2-5 mg sample f PNA-resin is taken out and dried thor ughly f r a quantitative ninhydrin analysis

to determine the substituti n; (6) addition f 2.5 equiv. (3.75 mmol; 2.064 g) BocTaeg-OPfp dissolved in 35 ml CH_2Cl_2 (final concentration of pentafluorophenylester ~0.1 M); the coupling reaction was allowed to proceed for a total of 20-24 h with shaking; (7) washing with DMF, 100 ml, 1 x 2 min (to remove precipitate of BocTaeg-OH); (8) washing with CH_2Cl_2, 100 ml, 4 x 1 min; (9) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 100 ml, 2 x 2 min; (10) washing with CH_2Cl_2, 100 ml, 6 x 1 min; (11) 2-5 mg sample of protected PNA-resin is taken out for a rapid qualitative ninhydrin test and a further 2-5 mg is dried thoroughly for a quantitative ninhydrin analysis to determine the extent of coupling; (12) blocking of unreacted amino groups by acetylation with a 100 ml mixture of acetic anhydride / pyridine / CH_2Cl_2 (1:1:2, v/v/v) for 2 h; (13) washing with CH_2Cl_2, 100 ml, 6 x 1 min; (14) 2 x 2-5 mg samples of protected PNA-resin are taken out, neutralized with DIEA/CH_2Cl_2 (1: 19, v/v) and washed with CH_2Cl_2 for qualitative and quantitative ninhydrin analyses.

(i) Synthetic Protocol 4.

(1) Boc-deprotection with TFA/CH_2Cl_2 (1:1, v/v), 25 ml, 3 x 1 min and 1 x 30 min; (2) washing with CH_2Cl_2, 25 ml, 6 x 1 min; (3) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 25 ml, 3 x 2 min; (4) washing with CH_2Cl_2, 25 ml, 6 x 1 min, and drain for 1 min; (5) 2-5 mg sample of PNA-resin is taken out and dried thoroughly for a quantitative ninhydrin analysis to determine the substitution; (6) addition of 2.5 equiv. (0.92 mmol; 0.506 g) BocTaeg-OPfp dissolved in 6 ml CH_2Cl_2 followed by addition of 3 ml DMF (final concentration of pentafluorophenylester ~0.1 M); the coupling reaction was allowed to proceed for a total of 20-24 hrs with shaking; (7) washing with DMF, 25 ml, 1 x 2 min; (8) washing with CH_2Cl_2, 25 ml, 4 x 1 min; (9) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 25 ml, 2 x 2 min; (10) washing with CH_2Cl_2, 25 ml, 6 x 1 min; (11) 2-5 mg sample f pr tected PNA-resin is taken out for a rapid qualitative ninhydrin test and a further 2-5 mg is dried th r ughly for a quantitative ninhydrin analysis t determine the extent of coupling; (12) blocking of unreacted

amino groups by ac tylation with a 25 ml mixture of acetic anhydride/pyridine/CH_2Cl_2 (1:1:2, v/v/v) f r 2 h (except after the first cycle); (13) washing with CH_2Cl_2, 25 ml, 6 x 1 min; (14) 2 x 2-5 mg samples of protected PNA-resin are taken out, neutralized with DIEA/CH_2Cl_2 (1: 19, v/v) and washed with CH_2Cl_2 for qualitative and quantitative ninhydrin analyses.

EXAMPLE 20

Solid-Phase Synthesis of H-[Taeg]$_5$-Caeg-[Taeg]$_4$-Lys-NH$_2$

(a) Stepwise Assembly of Boc-[Taeg]$_5$-C(z)aeg-[Taeg]$_4$-Lys(ClZ)-MBHA Resin

About 2.5 g of wet Boc-[Taeg]$_3$-Lys(ClZ)-MBHA resin (~ 1/6 of the total remaining about 16 g wet resin; ~0.75 g dry resin ~0.15 mmol growing chains) was placed in a 6 ml SPPS reaction vessel. Boc-[Taeg]$_5$-Caeg-[Taeg]$_4$-Lys(ClZ)-MBHA resin was assembled by double coupling of all Taeg-residues utilizing the usual 2.5 equivalents of BocTaeg-OPfp in 2.5 ml about 30% DMF/CH_2Cl_2, except that the first residue was single-coupled. Incorporation of the C(Z)aeg-residue was accomplished by coupling with 2.0 equivalents of BocC(Z)aeg-OPfp in TFE/CH_2Cl_2 (1:2, v/v). The progress of the synthesis was monitored at all stages by the quantitative ninhydrin reaction (see Table III).

Synthetic Step	Residue Coupled	Substitution After Deprotection (mmol/g)		Remaining Free Amino Groups After (μmol/g)			Estimated Extent of Coupling
		Measd.	Theoret.	1st Coupl	2nd Coupl	Acetylation	
3		0.19	0.23	1.00		0.57	
4	BocTaeg	0.17	0.21	4.88		97.3	97.3
5	BocC(Z)aeg	0.11	0.20	70.20	27.98	1.33	78.4 (46)
6	BocTaeg	0.10	0.19	24.79	4.58	2.40	95.4 (75)
7	BocTaeg	0.09	0.18	8.55	1.61	0.20	>99.9 (93)
8	BocTaeg	0.08	0.17	6.53	0.80	0.45	99.0 (91)
9	BocTaeg	0.07	0.16	9.26	3.66	0.61	94.8 (86)
10	BocTaeg	0.07	0.15	5.32	1.48	0.60	98.8 (93)

(b) Cleavag , Purification, and Identification f H-[Ta g]$_5$-Ca g-[Taeg]$_4$-Lys-NH_2

A portion of protected Boc-[Taeg]$_5$-Caeg-[Taeg]$_4$-Lys(ClZ)-BHA resin was treated as described in Example I section (c) to yield about 14.4 mg of crude material upon HF cleavage of 66.9 mg dry H-[Taeg]$_5$-Caeg-[Taeg]$_4$-Lys(ClZ)-BHA resin. The main peak at 14.5 min accounted for >50% of the total absorbance. A 100.0 mg portion of the crude product was purified (8 batches; each dissolved in 1 ml H_2O) to give approximately 9.1 mg of 96% pure H-[Taeg]$_5$-Caeg-[Taeg]$_4$-Lys-NH_2 (Figure 13b). For $(M+H)^+$ the calculated m/z value = 2793,8 and the measured m/z value = 2790,6.

EXAMPLE 21

Binding of Acr^1-(Taeg)$_{10}$-Lys-NH_2 to dA_{10} (Figure 11a)

Acr^1-(Taeg)$_{10}$-Lys (100 ng) was incubated for 15 min at room temperature with 50 cps 5'-[^{32}P]-end-labelled oligonucleotide [d(GATCCA$_{10}$G)] in 20 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4). The sample was cooled in ice (15 min) and analyzed by gel electrophoresis in polyacrylamide (PAGE). To 10 µl of the sample was added 2 µl 50% glycerol, 5 TBE (TBE = 90 mM Tris-borate, 1 mM EDTA, pH 8.3), and the sample was analysed by PAGE (15% acrylamide, 0.5% bisacrylamide) in TBE buffer at 4°C. A 10 µl portion of the sample was lyophilized and redissolved in 10 µl 80% formamide, 1 TBE, heated to 90°C (5 min), and analyzed by urea/PAGE (15% acrylamide, 0.5% bisacrylamide, 7 M urea) in TBE. [^{32}P]-containing DNA bands were visualized by autoradiography using intensifying screens and Agfa Curix RPI X-ray films exposed at -80°C for 2 h.

Oligonucleotides were synthesized on a Biosearch 7500 DNA synthesizer, labelled with γ[^{32}P]-ATP (Amersham, 5000 Ci/mmol) and polynucleotide kinase, and purified by PAGE using standard techniques (Maniatis et al. (1986): A laboratory manual, C ld Spring Harb r Laboratories).

EXAMPLE 22

Formation f strand displacement c mplex

A dA_{10}-dT_{10} target sequence contained within a plasmid DNA sequence was constructed by cloning of two oligonucleotides (d(GATCCA$_{10}$G) + d(GATCCT$_{10}$G)) into the *BamHI* restriction enzyme site of pUC19 using the Eschericia coli JM101 strain by standard techniques (Maniatis et al., 1986). The desired plasmid (designated pT10) was isolated from on of the resulting clones and purified by the alkalin extraction procedure and CsCl centrifugation (Maniatis et al., 1986). A 3'-[^{32}P]-end-labelled DNA fragment of 248 bp containing the dA_{10}/dT_{10} target sequence was obtained by cleaving the pT10 DNA with restriction enzymes EcoRI and PvuII, labelling of the cleaved DNA with α[^{32}P]-dATP (4000 Ci/mmol, Amersham) using the Klenow fragment of E. coli DNA polymerase (Boehringer Mannheim), and purifying the 248 bp DNA fragment by PAGE (5% acrylamide, 0.06% bisacrylamide, TBE buffer). This DNA fragment was obtained with [^{32}P]-end-labelling at the 5'-end by treating the EcoRI-cleaved pT10 plasmid with bacterial alkaline phosphatase (Boehringer Mannheim), purifying the plasmid DNA by gel electrophoresis in low melting agarose, and labelling with γ[^{32}P] ATP and polynucleotide kinase. Following treatment with PvuII, the 248 bp DNA fragment was purified as above.

The complex between Acr^1-(Taeg)$_{10}$-Lys-NH_2 and the 248 bp DNA fragment was formed by incubating 50 ng of Acr^1-(Taeg)$_{10}$-Lys-NH_2 with 500 cps ^{32}P-labelled 248 bp fragment and 0.5 μg calf thymus DNA in 100 μl buffer for 60 min at 37°C.

EXAMPLE 23

Probing of strand displacement complex with:

(a) Staphylococcus nuclease (Figure 12b)

The strand displacement complex was formed in 25 mM Tris-HCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, pH 7.4 as described above. The comples was treated with Staphyl coccus nuclease (B ehringer Mannheim) at 750 U/ml f r 5 min at 20°C and the reacti n was stopped by addition of EDTA t 25 mM. The DNA

was precipitated with 2 vols. of ethanol, 2% potassium acetate redissolved in 80% formamide, TBE, heated t 90°C (5 min), and analyzed by high resolution PAGE (10% acrylamide, 0.3% bisacrylamide, 7 M urea) and autoradiography.

(b) Affinity photocleavage (Figure 12a + 12b)

The complex was formed in TE buffer. A sample contained in an Eppendorf tube was irradiated from above at 300 nm (Philips TL 20 W/12 fluorescent light tube, 24 $Jm^{-2}s^{-1}$) for 30 min. The DNA was precipitated as above, taken up in 1 M piperidine, and heated to 90°C for 20 min. Following lyophilization, the DNA was analysed by PAGE as above.

(c) Potassium permanganate (Figure 12b)

The complex was formed in 100 µl TE and 5 µl 20 mM $KMnO_4$ was added. After 15 s at 20°C, the reaction was stopped by addition of 50 µl 1.5 M sodium acetate, pH 7.0, 1 M 2-mercaptoethanol. The DNA was precipitated, treated with piperidine and analyzed, as above.

(d) Photofootprinting (Figure 12b)

The complex was formed in 100 µl TE and diazo-linked acridine (0.1 µg/µl) (DHA, Nielsen et al. (1988) Nucl. Acids Res. 16, 3877-88) was added. The sample was irradiated at 365 nm (Philips TL 20 W/09N, 22 $Jm^{-2}s^{-1}$) for 30 min and treated as described for "affinity photocleavage".

(e) S_1-nuclease (Figure 12c)

The complex was formed in 50 mM sodium acetate, 200 mM NaCl, 0.5% glycerol, 1 mM $ZnCl_2$, pH 4.5 and treated with nuclease S_1 (Boehringer Mannheim) at 0.5 U/ml for 5 min at 20°C. The reaction was stopped and treated further as described under "Staphylococcus nuclease".

EXAMPLE 24

N-Benzyloxycarbonyl-N-'(bocaminoethyl)glycine.

Aminoethyl glycine (52.86 g; 0.447 mol) was dissolved in water (900 ml) and dioxane (900 ml) was added. Th pH was adjusted to 11.2 with 2N NaOH. While the pH was kept at 11.2, *tert*-butyl-p-nitrophenyl carbonate (128.4 g; 0.537 mol) was dissolved in dioxane (720 ml) and added dropwise over th

c urse f 2 h urs. Th pH was kept at 11.2 for at l ast three m re h urs and then left with stirring overnight. Th yellow solution was cooled to 0°C and the pH was adjusted to 3.5 with 2 N HCl. The mixture was washed with chloroform (4x100 ml), and the pH of the aqueous phase was readjusted to 9.5 with 2 N NaOH at 0°C. Benzyloxycarbonyl chloride (73.5 ml; 0.515 mol) was added over half an hour, while the pH was kept at 9.5 with 2 N NaOH. The pH was adjusted frequently over the next 4 hours, and the solution was left with stirring overnight. On the following day the solution was washed with ether (3x600 ml) and the pH of the solution was afterwards adjusted to 1.5 with 2 N HCl at 0°C. The title compound was isolated by extraction with ethyl acetate (5x1000 ml). The ethyl acetate solution was dried over magnesium sulfate and evaporated t dryness, *in vacuo*. This afforded 138 g, which was dissolved in ether (300 ml) and precipitated by the addition of petroleum ether (1800 ml). Yield 124.7 g (79%). M.p. 64.5-85 °C. Anal. for $C_{17}H_{24}N_2O_6$ found(calc.) C: 58.40(57.94); H: 7.02(6.86); N: 7.94(7.95). ^{1}H-NMR (250 MHz, $CDCl_3$) 7.33 & 7.32 (5H, Ph); 5.15 & 5.12 (2H, $PhC\underline{H_2}$); 4.03 & 4.01 (2H, $NC\underline{H_2}CO_2H$); 3.46 (b, 2H, $BocNHCH_2C\underline{H_2}$); 3.28 (b, 2H, $BocNHC\underline{H_2}CH_2$); 1.43 & 1.40 (9H, ^{t}Bu). HPLC (260 nm) 20.71 min. (80.2%) and 21.57 min. (19.8%). The UV-spectra (200 nm - 300 nm) are identical, indicating that the minor peak consists of Bis-Z-AEG.

EXAMPLE 25

N'-Boc-aminoethyl glycine ethyl ester.

N-Benzyloxycarbonyl-N'-(bocaminoethyl)glycine (60.0 g; 0.170 mol) and N,N-dimethyl-4-aminopyridine (6.00 g) were dissolved in absolute ethanol (500 ml), and cooled to 0°C before the addition of DCC (42.2 g; 0.204 mol). The ice bath was removed after 5 minutes and stirring was continued for 2 more hours. The precipitated DCU (32.5 g dried) was removed by filtration and washed with ether (3x100 ml). The combin d filtrate was washed successively with diluted p tassium hydr gen sulfate (2x400 ml), diluted s dium hydrogencarb nate (2x400 ml) and saturated s dium chl ride (1x400 ml). The

organic phase was filter d, th n dried over magnesium sulfate, and evaporated to dryness, *in vacuo*, which yielded 66.1 g f an oily substance which contained some DCU.

The oil was dissolved in absolute ethanol (600 ml) and was added 10% palladium on carbon (6.6 g) was added. The solution was hydrogenated at atmospheric pressure, where the reservoir was filled with 2 N sodium hydroxide. After 4 hours, 3.3 L was consumed out of the theoretical 4.2 L. The reaction mixture was filtered through celite and evaporated to dryness, *in vacuo*, affording 39.5 g (94%) of an oily substance. A 13 g portion of the oily substance was purified by silica gel (600 g SiO_2) chromatography. After elution with 300 ml 20% petroleum ether in methylene chloride, the title compound was eluted with 1700 ml of 5% methanol in methylene chloride. The solvent was removed from the fractions with satisfactory purity, *in vacuo* and the yield was 8.49 g. Alternatively 10 g of the crude material was purified by Kugel Rohr distillation. ^{1}H-NMR (250 MHz, CD_3OD); 4.77 (b. s, NH); 4.18 (q, 2H, Me$\underline{CH_2}$-); 3.38 (s, 2H, N$\underline{CH_2}CO_2$Et); 3.16 (t, 2H, BocNH$\underline{CH_2}CH_2$); 2.68 (t, 2H, BocNH$CH_2\underline{CH_2}$); 1.43 (s, 9H, ^{t}Bu) and 1.26 (t, 3H, CH_3) ^{13}C-NMR 171.4 ($\underline{C}$OEt); 156.6 (CO); 78.3 ($(CH_3)_3\underline{C}$); 59.9 (CH_2); 49.0 (CH_2); 48.1 (CH_2); 39.0 (CH_2); 26.9 (CH_2) and 12.6 (CH_3).

EXAMPLE 26

N'-Boc-aminoethyl glycine methyl ester.

The above procedure was used, with methanol being substituted for ethanol. The final product was purified by column purification.

EXAMPLE 27

1-(Boc-aeg)thymine ethyl ester.

N'-B c-aminoethyl glycine ethyl ester (13.5 g; 54.8 mmol), DhbtOH (9.84 g; 60.3 mmol) and 1-carboxymethyl thymine (11.1 g; 60.3 mm l) were diss lved in DMF (210 ml). Methylene chloride (210 ml) then was added. The solution was cooled to 0°C in an ethanol/ice bath and DCC (13.6 g; 65.8

mm l) was added. The ice bath was removed after 1 h ur and stirring was continued for another 2 hours at ambient temperature. The precipitated DCU was removed by filtration and washed twice with methylene chloride (2 x 75 ml). To th combined filtrate was added more methylene chloride (650 ml). The solution was washed successively with diluted sodium hydrogen carbonate (3 x 500 ml), diluted potassium hydrogen sulfate (2 x 500 ml), and saturated sodium chloride (1 x 500 ml). Some precipitate was removed from the organic phase by filtration, The organic phase was dried over magnesium sulfate and evaporated to dryness, *in vacuo*. The oily residu was dissolved in methylene chloride (150 ml), filtered, and the title compound was precipitated by the addition of petroleum ether (350 ml) at 0°C. The methylene chloride/petroleum ether procedure was repeated once. This afforded 16.0 g (71%) of a material which was more than 99% pure by HPLC.

EXAMPLE 28

1-(Boc-aeg)thymine.

The material from above was suspended in THF (194 ml, gives a 0.2 M solution), and 1 M aqueous lithium hydroxide (116 ml) was added. The mixture was stirred for 45 minutes at ambient temperature and then filtered to remove residual DCU. Water (40 ml) was added to the solution which was then washed with methylene chloride (300 ml). Additional water (30 ml) was added, and the alkaline solution was washed once more with methylene chloride (150 ml). The aqueous solution was cooled to 0°C and the pH was adjusted to 2 by the dropwise addition of 1 N HCl (approx. 110 ml). The title compound was extracted with ethyl acetate (9 x 200 ml), the combined extracts were dried over magnesium sulfate and were evaporated to dryness, *in vacuo*. The residue was evaporated once from methanol, which after drying overnight afforded a colorless glassy s lid. Yield 9.57 g (64 %). HPLC > 98% R_T=14.8 min. Anal. f r $C_{16}H_{24}N_4O_7$°0.25 H_2O F und (calc.) C: 49.29(49.42); H: 6.52(6.35); N: 14.11(14.41). Due to th limited rotation

around th secondary amide, several of the signals wer doubled in the ratio 2:1 (indicated in the list by mj. for major and mi. for minor). ^{1}H-NMR (250 MHz, DMSO-d_6): 12.75 (b.s., 1H, CO_2H); 11.28 (s, "1H", mj., imide NH); 11.26 (s, "1H", mi., imide NH); 7.30 (s, "1H", mj., T H-6); 7.26 (s, "1H", mi., T H-6); 6.92 (b.t., "1H", mj., BocNH); 6.73 (b.t., "1H", mi., BocNH); 4.64 (s, "2H", mj., $C\underline{H}_2CON$); 4.46 (s, "2H", mj., $C\underline{H}_2CON$); 4.19 (s, "2H", mi., $C\underline{H}_2CO_2H$); 3.97 (s, "2H", mj., $C\underline{H}_2CO_2H$); 3.63-3.01 (unresolved m, includes water, $C\underline{H}_2C\underline{H}_2$); 1.75 (s, 3H, $C\underline{H}_3$) and 1.38 (s, 9H, ^{t}Bu).

EXAMPLE 29

N^4-Benzyloxycarbonyl-1-(Boc-aeg)cytosine.

N′-Boc-aminoethyl glycine ethyl ester (5.00 g; 20.3 mmol), DhbtOH (3.64 g; 22.3 mmol) and N^4-benzyloxycarbonyl-1-carboxymethyl cytosine (6.77 g; 22.3 mmol) were suspended in DMF (100 ml). Methylene chloride (100 ml) then was added. The solution was cooled to 0°C and DCC (5.03 g; 24.4 mmol) was added. The ice bath was removed after 2 h and stirring was continued for another hour at ambient temperature. The reaction mixture then was evaporated to dryness, *in vacuo*. The residue was suspended in ether (100 ml) and stirred vigorously for 30 min. The solid material was isolated by filtration and the ether wash procedure was repeated twice. The material was then stirred vigorously for 15 min with dilute sodium hydrogencarbonate (aprox. 4% solution, 100 ml), filtered and washed with water. This procedure was then repeated once, which after drying left 17.0 g of yellowish solid material. The solid was then boiled with dioxane (200 ml) and filtered while hot. After cooling, water (200 ml) was added. The precipitated material was isolated by filtration, washed with water, and dried. According to HPLC (observing at 260 nm) this material has a purity higher than 99%, besides the DCU. The ester was then suspended in THF (100 ml), c oled t 0°C, and 1 N LiOH (61 ml) was added. After stirring for 15 minutes, the mixture was filtered and the filtrate was washed with methylene chl ride (2 x 150 ml). The alkaline

solution then was c oled to 0°C and th pH was adjusted to 2.0 with 1 N HCl. The title compound was is lated by filtrati n and was washed once with water, leaving 11.3 g of a whit powder after drying. The material was suspended in methylene chloride (300 ml) and petroleum ether (300 ml) was added. Filtration and wash afforded 7.1 g (69%) after drying. HPLC showed a purity of 99% R_T= 19.5 min, and a minor impurity at 12.6 min (approx. 1%) most likely the Z-de protected monomer. Anal. for $C_{23}H_{29}N_5O_8$ found(calc.) C: 54.16(54.87); H: 5.76(5.81) and N: 13.65(13.91). ^{1}H-NMR (250 MHz, DMSO-d_6). 10.78 (b.s, 1H, CO$_2$<u>H</u>); 7.88 (2 overlapping dublets, 1H, Cyt H-5); 7.41-7.32 (m, 5H, Ph); 7.01 (2 overlapping doublets, 1H, Cyt H-6); 6.94 & 6.78 (unres. triplets, 1H, BocN<u>H</u>); 5.19 (s, 2H, PhC<u>H</u>$_2$); 4.81 & 4.62 (s, 2H, C<u>H</u>$_2$CON); 4.17 & 3.98 (s, 2H, C<u>H</u>$_2$CO$_2$H); 3.42-3.03 (m, includes water, C<u>H</u>$_2$C<u>H</u>$_2$) and 1.38 & 1.37 (s, 9H, ^{t}Bu). ^{13}C-NMR. 150.88; 128.52; 128.18; 127.96; 93.90; 66.53; 49.58 and 28.22. IR: Frequency in cm^{-1} (intensity). 3423 (26.4), 3035 (53.2), 2978(41.4), 1736(17.3), 1658(3.8), 1563(23.0), 1501(6.8) and 1456 (26.4).

EXAMPLE 30

9-Carboxymethyl adenine ethyl ester.

Adenine (10.0 g, 74 mmol) and potassium carbonate (10.29 g, 74.0 mmol) were suspended in DMF and ethyl bromoacetate (8.24 ml, 74 mmol) was added. The suspension was stirred for 2.5 h under nitrogen at room temperature and then filtered. The solid residue was washed three times with DMF (10 ml). The combined filtrate was evaporated to dryness, *in vacuo*. The yellow-orange solid material was poured into water (200 ml) and 4 N HCl was added to pH≈6. After stirring at 0°C for 10 min, the solid was filtered off, washed with water, and recrystallized from 96% ethanol (150 ml). The title compound was isolated by filtration and washed thoroughly with ether. Yield 3.4 g (20%). M.p. 215.5-220°C. Anal. for $C_9H_{11}N_5O_2$ found(calc.): C: 48.86(48.65); H: 5.01(4.91); N: 31.66(31.42). ^{1}H-NMR (250 MHz; DMSO-d_6): (s, 2H, H-2 & H-8), 7.25 (b. s., 2H, NH$_2$), 5.06 (s, 2H, NCH$_2$), 4.17 (q, 2H, J=7.11 Hz, OCH$_2$) and

1.21 (t, 3H, J=7.13 Hz, NCH_2). ^{13}C-NMR. 152.70, 141.30, 61.41, 43.97 and 14.07. FAB-MS. 222 (MH+). IR: Frequency in cm^{-1} (intensity). 3855 (54.3), 3274(10.4), 3246(14.0), 3117(5.3), 2989(22.3), 2940(33.9), 2876(43.4), 2753(49.0), 2346(56.1), 2106(57.1), 1899(55.7), 1762(14.2), 1742(14.2), 1742(1.0), 1671(1.8), 1644(10.9), 1606(0.6), 1582(7.1), 1522(43.8), 1477(7.2), 1445(35.8) and 1422(8.6). The position of alkylation was verified by X-ray crystallography on crystals, which were obtained by recrystallization from 96% ethanol.

Alternatively, 9-carboxymethyl adenine ethyl ester can be prepared by the following procedure. To a suspension of adenine (50.0 g, 0.37 mol) in DMF (1100 ml) in 2 L three-necked flask equipped with a nitrogen inlet, a mechanical stirrer and a dropping funnel was added 16.4 g (0.407 mol) haxane washed sodium hydride- mineral oil dispersion. The mixture was stirred vigorously for 2 hours, whereafter ethy bromacetate 75 ml, 0.67 mol) was added dropwise over the course of 3 hours. The mixture was stirred for one additional hour, whereafter tlc indicated complete conversion of adenine. The mixture was evaporated to dryness at 1 mmHg and water (500 ml) was added to the oily residue which caused crystallisation of the title compound. the solid was recrystallised from 06% ethanol (600 ml). Yield after drying 53.7 (65.6%). HPLC (215 nm) purity > 99.5%.

EXAMPLE 31

N^6Benzyloxycarbonyl-9-carboxymethyl adenine ethyl ester.

9-Carboxymethyladenine ethyl ester (3.40 g, 15.4 mmol) was dissolved in dry DMF (50 ml) by gentle heating, cooled to 20°C, and added to a solution of N-ethyl- benzyloxycarbonylimidazole tetrafluoroborate (62 mmol) in methylene chloride (50 ml) over a period of 15 min with ice-cooling. Some precipitation was observed. The ice bath was remov d and the soluti n was stirred vernight. The r action mixture was treated with saturated s dium hydrogen carbonate (100 ml). After stirring for 10 min, the phases wer separated and the organic phase was washed successively with

on v lume of water, dilute potassium hydr gen sulfate (twice), and with saturated sodium chl ride. The s lution was dried over magnesium sulfate and evaporated to dryness, *in vacuo*, which afforded 11 g of an oily material. The material was dissolved in methylene chloride (25 ml), cooled to 0°C, and precipitated with petroleumeum ether (50 ml).. This procedure was repeated once to give 3.45 g (63%) of the title compound. M.p. 132-35°C. Analysis for $C_{17}H_{17}N_5O_4$ found (calc.): C: 56.95(57.46); H: 4.71(4.82); N: 19.35(19.71). ^{1}H-NMR (250 MHz; $CDCl_3$): 8.77 (s, 1H, H-2 or H-8); 7.99 (s, 1H, H-2 or H-8); 7.45-7.26 (m, 5H, Ph); 5.31 (s, 2H, N-C$\underline{H}_2$); 4.96 (s, 2H, Ph-C$\underline{H}_2$); 4.27 (q, 2H, J=7.15 Hz, C$\underline{H}_2$CH$_3$) and 1.30 (t, 3H, J=7.15 Hz, CH$_2$C$\underline{H}_3$). ^{13}C-NMR: 153.09; 143.11; 128.66; 67.84; 62.51; 44.24 and 14.09. FAB-MS: 356 (MH+) and 312 (MH+-CO_2). IR: frequency in cm^{-1} (intensity). 3423 (52.1); 3182 (52.8); 3115(52.1); 3031(47.9); 2981(38.6); 1747(1.1); 1617(4.8); 15.87(8.4); 1552(25.2); 1511(45.2); 1492(37.9); 1465(14.0) and 1413(37.3).

EXAMPLE 32

N^6-Benzyloxycarbonyl-9-carboxymethyl adenine.

N^6-Benzyloxycarbonyl-9-carboxymethyladenine ethyl ester (3.20 g; 9.01 mmol) was mixed with methanol (50 ml) cooled t 0°C. Sodium Hydroxide Solution (50 ml; 2N) was added, whereby the material quickly dissolved. After 30 min at 0°C, the alkaline solution was washed with methylene chloride (2x50ml). The aqueous solution was brought to pH 1.0 with 4 N HCl at 0°C, whereby the title compound precipitated. The yield after filtration, washing with water, and drying was 3.08 g (104%). The product contained salt and elemental analysis reflected that. Anal. for $C_{15}H_{13}N_5O_4$ found(calc.): C: 46.32(55.05); H: 4.24(4.00); N: 18.10(21.40) and C/N: 2.57(2.56). ^{1}H-NMR(250 MHz; DMSO-d_6): 8.70 (s, 2H, H-2 and H-8); 7.50-7.35 (m, 5H, Ph); 5.27 (s, 2H, N-C$\underline{H}_2$); and 5.15 (s, 2H, Ph-C$\underline{H}_2$). ^{13}C-NMR. 168.77, 152.54, 151.36, 148.75, 145.13, 128.51, 128.17,127.98, 66.76 and 44.67.IR (KBr) 3484(18.3); 3109(15.9); 3087(15.0); 2966(17.1); 2927(19.9); 2383(53.8); 1960(62.7); 1739(2.5);

1688(5.2); 1655(0.9); 1594(11.7); 1560(12.3); 1530(26.3); 1499(30.5); 1475(10.4); 1455(14.0); 1429(24.5) and 1411(23.6). FAB-MS: 328 (MH+) and 284 (MH+-CO_2). HPLC (215 nm, 260 nm) in system 1: 15.18 min, minor impurities all less than 2%.

EXAMPLE 33

N^6-Benzyloxycarbonyl-1-(Boc-aeg)adenine ethyl ester.

N′-Boc-aminoethyl glycine ethyl ester (2.00 g; 8.12 mmol), DhbtOH (1.46 g; 8.93 mmol) and N^6-benzyloxycarbonyl-9-carboxymethyl adenine (2.92 g; 8.93 mmol) were dissolved in DMF (15 ml). Methylene chloride (15 ml) then was added. The solution was cooled to 0°C in an ethanol/ice bath. DCC (2.01 g; 9.74 mmol) was added. The ice bath was removed after 2.5 h and stirring was continued for another 1.5 hour at ambient temperature. The precipitated DCU was removed by filtration and washed once with DMF (15 ml), and twice with methylene chloride (2 x 15 ml). To the combined filtrate was added more methylene chloride (100 ml). The solution was washed successively with dilute sodium hydrogen carbonate (2 x 100 ml), dilute potassium hydrogen sulfate (2 x 100 ml), and saturated sodium chloride (1 x 100 ml). The organic phase was evaporated to dryness, *in vacuo*, which afforded 3.28 g (73%) of a yellowish oily substance. HPLC of the raw product showed a purity of only 66% with several impurities, both more and less polar than the main peak. The oil was dissolved in absolute ethanol (50 ml) and activated carbon was added. After stirring for 5 minutes, the solution was filtered. The filtrate was mixed with water (30 ml) and was left with stirring overnight. The next day, the white precipitate was removed by filtration, washed with water, and dried, affording 1.16 g (26%) of a material with a purity higher than 98% by HPLC. Addition of water to the mother liquor afforded another 0.53 g with a purity of approx. 95%. Anal. for $C_{26}H_{33}N_7O_7 \cdot H_2O$ f und(calc.) C: 55.01(54.44; H: 6.85(6.15) and N: 16.47(17.09). ^{1}H-NMR (250 MHz, $CDCl_3$) 8.74 (s, 1H, Ade H-2); 8.18 (b. s, 1H, ZNH); 8.10 & 8.04 (s, 1H, H-8); 7.46-7.34 (m, 5H, Ph); 5.63 (unres. t, 1H, BocNH); 5.30 (s, 2H, $PhCH_2$); 5.16

& 5.00 (s, 2H, C$\underline{H}_2$CON); 4.29 & 4.06 (s, 2H, C$\underline{H}_2$CO$_2$H); 4.20 (q, 2H, OC$\underline{H}_2$CH$_3$); 3.67-3.29 (m, 4H, C$\underline{H}_2$C$\underline{H}_2$); 1.42 (s, 9H, ^{t}Bu) and 1.27 (t, 3H, OCH$_2$C$\underline{H}_3$). The spectrum shows traces of ethanol and DCU.

EXAMPLE 34

N^6-Benzyloxycarbonyl-1-(Boc-aeg)adenine.

N^6-Benzyloxycarbonyl-1-(Boc-aeg)adenine ethyl ester (1.48 g; 2.66 mmol) was suspended in THF (13 ml) and th mixture was cooled to 0°C. Lithium hydroxide (8 ml; 1 N) was added. After 15 min of stirring, the reaction mixture was filtered, extra water (25 ml) was added, and the solution was washed with methylene chloride (2 x 25 ml). The pH of the aqueous solution was adjusted to pH 2.0 with 1 N HCl. The precipitate was isolated by filtration, washed with water, and dried, and drief affording 0.82 g (58%). The product reprecipitated twice with methylene chloride/petroleum ether, 0.77 g (55%) after drying. M.p. 119°C (decomp.) Anal. for $C_{24}H_{29}N_7O_7$°H_2O found(calc.) C: 53.32(52.84); H: 5.71(5.73); N: 17.68(17.97). FAB-MS. 528.5 (MH+). ^{1}H-NMR (250 MHz, DMSO-d_6). 12.75 (very b, 1H, CO$_2$H); 10.65 (b. s, 1H, ZNH); 8.59 (d, 1H, J= 2.14 Hz, Ade H-2); 8.31 (s, 1H, Ade H-8); 7.49-7.31 (m, 5H, Ph); 7.03 & 6.75 (unresol. t, 1H, BocNH); 5.33 & 5.16 (s, 2H, CH$_2$CON); 5.22 (s, 2H, PhC$\underline{H}_2$); 4.34-3.99 (s, 2H, CH$_2$CO$_2$H); 3.54-3.03 (m's, includes water, C$\underline{H}_2$C$\underline{H}_2$) and 1.39 & 1.37 (s, 9H, ^{t}Bu). ^{13}C-NMR. 170.4; 166.6; 152.3; 151.5; 149.5; 145.2; 128.5; 128.0; 127.9; 66.32; 47.63; 47.03; 43.87 and 28.24.

EXAMPLE 35

2-Amino-6-chloro-9-carboxymethylpurine.

To a suspension of 2-amino-6-chloropurine (5.02 g; 29.6 mmol) and potassium carbonate (12.91 g; 93.5 mmol) in DMF (50 ml) was added bromoacetic acid (4.70 g; 22.8 mmol). The mixture was stirr d vigorously for 20 h. und r nitr gen. Water (150 ml) was added and the s luti n was filtered through Celite to give a clear yellow s lution. The solution was acidified t a pH f 3 with 4 N hydrochl ric acid. Th

pr cipitate was filtered and dried, *in vacuo*, ov r sicapent. Yield (3.02 g; 44.8%). ^{1}H-NMR(DMSO-d6): d = 4.88 ppm (s,2H); 6.95 (s,2H); 8.10 (s,1H).

EXAMPLE 36

2-Amino-6-benzyloxy-9-carboxymethylpurine.

Sodium (2.0 g; 87.0 mmol) was dissolved in benzyl alcohol (20 ml) and heated to 130°C for 2 h. After cooling to 0°C, a solution of 2-amino-6-chloro-9-carboxymethylpurine (4.05 g; 18.0 mmol) in DMF (85 ml) was slowly added, and the resulting suspension stirred overnight at 20°C. Sodium hydroxide solution (1N, 100 ml) was added and the clear solution was washed with ethyl acetate (3 x 100 ml). The water phase then was acidified to a pH of 3 with 4 N hydrochloric acid. The precipitate was taken up in ethyl acetate (200 ml), and the water phase was extracted with ethyl acetate (2 x 100 ml). The combined organic phases were washed with saturated sodium chloride solution (2 x 75 ml), dried with anhydrous sodium sulfate, and taken to dryness by evaporation, *in vacuo*. The residue was recrystallized from ethanol (300 ml). Yield after drying, *in vacou*, over sicapent: 2.76 g (52%). M.p. 159-65°C. Anal. (calc., found) C(56.18; 55.97), H(4.38; 4.32), N(23.4; 23.10). ^{1}H-NMR (DMSO-d_6): 4.82 ppm.(s,2H); 5.51 (s,2H); 6.45 (s,2H); 7.45 (m,5H); 7.82 (s,1H).

EXAMPLE 37

N-([2-Amino-6-benzyloxy-purine-9-yl]-acetyl)-N-(2-Boc-aminoethyl)-glycine [BocGaeg-OH monomer].

2-Amino-6-benzyloxy-9-carboxymethyl-purine (0.50 g; 1.67 mmol), methyl-N(2-[*tert*-butoxycarbonylamino]ethyl)-glycinate (0.65 g; 2.80 mmol), diisopropylethyl amine (0.54 g; 4.19 mmol), and bromo-tris-pyrrolidino-phosphonium-hexafluoro-phosphate (PyBroP) (0.798 g; 1.71 mmol) wer stirred in DMF (2 ml) for 4 h. The clear soluti n was p ured into an ice-cool d solution of s dium hydrogen carb nat (1 N; 40 ml) and extracted with ethyl acetate (3 X 40 ml). The

organic layer was washed with potassium hydrogen sulfate s lution (1 N; 2 X 40 ml), sodium hydrogen carbonat (1 N; 1 X 40 ml) and saturated sodium chloride solution (60 ml). After drying with anhydrous sodium sulfate and evaporation, *in vacuo,* the solid residue was recrystallized from ethyl acetate/hexane (20 ml (2:1)) to give the methyl ester in 63% yield (MS-FAB 514 (M+1). Hydrolysis was accomplished by dissolving the ester in ethanol/water (30 ml (1:2)) containing conc. sodium hydroxide (1 ml). After stirring for 2 h, th solution was filtered and acidified to a pH of 3, by the addition of 4 N hydrochloric acid. The title compound was obtained by filtration. Yield: 370 mg (72% for th hydrolysis). Purity by HPLC was more than 99%. Due to th limited rotation around the secondary amide several of th signals were doubled in the ratio 2:1 (indicated in the list by mj. for major and mi. for minor). ^{1}H-NMR(250, MHz, DMSO-d_6): d = 1.4 ppm. (s,9H); 3.2 (m,2H); 3.6 (m,2H); 4.1 (s, mj., CONRCH_2COOH); 4.4 (s, mi., CONRCH_2COOH); 5.0 (s, mi., Gua-CH_2CO-); 5.2 (s, mj., Gua-CH_2CO); 5.6 (s,2H); 6.5 (s,2H); 6.9 (m, mi., BocNH); 7.1 (m, mj., BocNH); 7.5 (m.,3H); 7.8 (s,1H); 12,8 (s;1H). ^{13}C-NMR. 170.95; 170.52; 167.29; 166.85; 160.03; 159.78; 155.84; 154.87; 140.63; 136.76; 128.49; 128.10; 113.04; 78.19; 77.86; 66.95; 49.22; 47.70; 46.94; 45.96; 43.62; 43.31 and 28.25.

EXAMPLE 38

3-Boc-amino-1,2-propanediol.

3-Amino-1,2-propanediol (40.00 g, 0.440 mol, 1.0 eq.) was dissolved in water (1000 ml) and cooled to 0°C. Di-tert-butyl dicarbonate (115.0 g, 0.526 mol, 1.2 eq.) was added in one portion. The reaction mixture was heated to ro m temperature on a water bath during stirring. The pH was maintained at 10.5 with a solution of sodium hydroxide (17.56 g, 0.440 mol, 1.0 eq.) in water (120 ml). When the addition of aqueous sodium hydroxid was c mpleted, the reacti n mixture was stirred overnight at r om temperature. Subsequently, thyl acetate (750 ml) was added to the r acti n

mixture, followed by cooling to 0°C. Th pH was adjusted to 2.5 with 4 N sulphuric acid with vig rous stirring. The phases were separated and the water phase was washed with additional ethyl acetate (6x350 ml). The volume of the organic phase was reduced to 900 ml by evaporation under reduced pressure. The organic phase then was washed.with a saturated aqueous solution of potassium hydrogen sulfate diluted to twice its volume (1x1000 ml) and with saturated aqueous sodium chloride (1x500 ml). The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to yield 50.12 g (60%) of the title compound. The product could be solidified by evaporation from methylene chloride and subsequent freezing. ^{1}H-NMR ($CDCl_3$/TMS): d = 1.43 (s, 9H, Me_3C), 3.25 (m, 2H, CH_2), 3.57 (m, 2H, CH_2), 3.73 (m, 1H, CH). ^{13}C-NMR ($CDCl_3$/TMS): d = 28.2 (Me_3C), 42.6 (CH_2), 63.5, 71.1 (CH_2OH, CHOH), 79.5 (Me_3C), 157.0 (C=O).

EXAMPLE 39

2-(Boc-amino)ethyl-L-alanine methyl ester.

3-Boc-amino-1,2-propanediol (20.76 g, 0.109 mol, 1 eq.) was suspended in water (150 ml). Potassium *m*-periodate (24.97 g, 0.109 mol, 1 eq.) was added and the reaction mixture was stirred for 2 h at room temperature under nitrogen. The reaction mixture was filtered and the water phase extracted with chloroform (6x250 ml) The organic phase was dried ($MgSO_4$) and evaporated to afford an almost quantitative yield of Boc-aminoacetaldehyde as a colourless oil, which was used without further purification in the following procedure.

Palladium-on-carbon (10%, 0.8 g) was added to MeOH (250 ml) under nitrogen with cooling (0°C) and vigorous stirring. Anhydrous sodium acetate (4.49 g, 54.7 mmol, 2 eqv) and L-alanine methyl ester, hydrochloride (3.82 g, 27.4 mmol, 1 eqv) were added. Boc-aminoacetaldehyde (4.79 g, 30.1 mmol, 1.1 eqv) was dissolved in MeOH (150 ml) and add d t the reaction mixture. The reaction mixture was hydrogenated at atmospheric pressure and r om temperature until hydrogen uptake had c ased. The reaction mixture was filtered through celite,

which was washed with additi nal MeOH. The MeOH was removed und r reduced pressure. The r sidue was suspended in water (150 ml) and pH adjusted to 8.0 by dropwise addition of 0.5 N NaOH with vigorous stirring. The water phase was extracted with methylene chloride (4x250 ml). The organic phase was dried ($MgSO_4$), filtered through celite, and evaporated under reduced pressure to yield 6.36 g (94%) of the title compound as a clear, slightly yellow oil. MS (FAB-MS): m/z (%) = 247 (100, M+1, 191 (90), 147 (18). ^{1}H-NMR (250 MHz, $CDCl_3$). 1.18 (d, J=7.0 Hz, 3H, Me), 1.36 (s, 9H, Me_3C), 1.89 (b, 1H, NH), 2.51 (m, 1H, CH_2), 2.66 (m, 1H, CH_2), 3.10 (m, 2H, CH_2), 3.27 (q, J=7.0 Hz, 1H, CH), 3.64 (s, 3H, OMe), 5.06 (b, 1H, carbamate NH). ^{13}C-NMR. d = 18.8 (Me), 28.2 (Me_3C), 40.1, 47.0 (CH_2), 51.6 (OMe), 56.0 (CH), 155.8 (carbamate C=O), 175.8 (ester C=O).

EXAMPLE 40

N-(Boc-aminoethyl)-N-(1-thyminylacetyl)-L-alanine methyl ester.

To a solution of Boc-aminoethyl-(L)-alanine methyl ester (1.23 g, 5.0 mmol) in DMF (10 ml) was added Dhbt-OH (0,90 g, 5.52 mmol) and 1-thyminylacetic acid (1.01 g, 5.48 mmol). When the 1-thyminylacetic acid was dissolved, dichloromethane (10 ml) was added and the solution was cooled on an ice bath. After the reaction mixture had reached 0°C, DCC (1.24 g, 6.01 mmol) was added. Within 5 min after the addition, a precipitate of DCU was seen. After a further 5 min, the ice bath was removed. Two hours later, TLC analysis showed the reaction to be finished. The mixture was filtered and the precipitate washed with dichloromethane (100ml). The resulting solution was extracted twice with 5% sodium hydrogen carbonate (150 ml) and twice with saturated potassium hydrogen sulfate (25 ml) in water (100 ml). After a final extraction with saturated sodium chloride (150 ml), the solution was dri d with magn sium sulfate and evap rated to give a white foam. The foam was purified by c lumn chromatography on silica gel using dichloromethane with a methan l gradient as

eluent. This yielded a pure comp und (>99% by HPLC) (1.08 g, 52.4%). FAB-MS: 413 (M+1) and 431 (M+1 + water). ^{1}H-NMR ($CDCl_3$): 4.52 (s, 2 H, CH´$_2$); 3,73 (s, 3 H, OMe); 3.2-3.6 (m, 4 H, ethyl CH_2´s); 1.90 (s, 3 H, Me in T); 1.49 (d, 3 H, Me in Ala, J=7.3 Hz); 1.44 (s, 9 H, Boc).

EXAMPLE 41

N-(Boc-aminoethyl)-N-(1-thyminylacetyl)-L-alanine.

The methyl ester of the title compound (2.07 g, 5.02 mmol) was dissolved in methanol (100 ml), and cooled on an ice bath. 2 M sodium hydroxide (100 ml) was added. After stirring for 10 min, the pH of the mixture was adjusted to 3 with 4 M hydrogen chloride. The solution was subsequently extracted with ethyl acetate (3 x 100 ml). The combined organic extracts were dried over magnesium sulfate. After evaporation, the resulting foam was dissolved in ethyl acetate (400 ml) and a few ml of methanol to dissolve the solid material. Petroleum ether then was added until precipitation started. After standing overnight at -20°C, the precipitate was removed by filtration. This gave 1.01 g (50.5%) of pure compound (>99% by HPLC). The compound can be recrystallized from 2-propanol. FAB-MS: 399 (M+1). ^{1}H-NMR (DMSO-d_6): 11.35 (s, 1 H, COO); 7.42 (s, 1 H, H´$_6$); 4.69 (s, 2 H, CH´$_2$); 1.83 (s, 3 H, Me in T); 1.50-1.40 (m, 12 H, Me in Ala + Boc).

EXAMPLE 42

(a) N-(Boc-aminoethyl)--N-(1-thyminylacetyl)-D-alanine methyl ester.

To a solution of Boc-aminoethyl alanine methyl ester (2.48 g, 10.1 mmol) in DMF (20 ml) was added Dhbt-OH (1.80 g, 11.0 mmol) and thyminylacetic acid (2.14 g, 11.6 mmol). After dissolution of the 1-thyminylacetic acid, methylene chloride (20 ml) was added and the solution cooled on an ice bath. When the reaction mixture had reached 0°C, DCC (2.88 g, 14.0 mmol) was added. Within 5 min after the addition a precipitate f DCU was seen. After 35 min the ice bath was rem ved. The reaction mixture was filtered 3.5 h later and

the precipitate washed with methyl n chl ride (200 ml). The resulting soluti n was extracted twice with 5% sodium hydrogen carbonate (200 ml) and twice with saturated potassium hydrogen sulfate in water (100 ml). After a final extraction with saturated sodium chloride (250 ml), the solution was dried with magnesium sulfate and evaporated to give an oil. The oil was purified by short column silica gel chromatography using methylene chloride with a methanol gradient as eluent. This yielded a compound which was 96% pure according to HPLC (1.05 g, 25.3%) after precipitation with petroleum ether. FAB-MS: 413 (M+1). ^{1}H-NMR ($CDCl_3$): 5.64 (t, 1 H, BocNH, J=5.89 Hz); 4.56 (d, 2 H, CH'_2); 4.35 (q, 1 H, CH in Ala, J=7.25 Hz); 3.74 (s, 3 H, OMe); 3.64-3.27 (m, 4 H, ethyl H's); 1.90 (s, 3 H, Me in T); 1.52-1.44 (t, 12 H, Boc+Me in Ala).

(b) N-(Boc-aminoethyl)-N-(1-thyminylacetyl)-D-alanine

The methyl ester of the title compound (1.57 g, 3.81 mmol) was dissolved in methanol (100 ml) and cooled on an ice bath. Sodium hydroxide (100 ml; 2 M) was added. After stirring for 10 min the pH of the mixture was adjusted to 3 with 4 M hydrogen chloride. The solution then was extracted with ethyl acetate (3 x 100 ml). The combined organic extracts were dried over magnesium sulfate. After evaporation, the oil was dissolved in ethyl acetate (200 ml). Petroleum ether was added (to a total volume of 600 ml) until precipitation started. After standing overnight at -20°C, the precipitate was removed by filtration. This afforded 1.02 g (67.3%) of the title compound, which was 94% pure according to HPLC. FAB-MS: 399 (M+1). ^{1}H-NMR: 11.34 (s, 1 H, COOH); 7.42 (s, 1 H, H'_6); 4.69 (s, 2 H, CH'_2); 4.40 (q, 1 H, CH in Ala, J=7.20 Hz); 1.83 (s, 3 H, Me in T); 1.52-1.40 (m, 12 H, Boc + Me in Ala).

EXAMPLE 43

N-(N'-Boc-3'-aminopropyl)-N-[(1-thyminyl)acetyl]glycine methyl ster.

N-(N'-Boc-3'-aminopropyl)glycine methyl ster (2.84 g, 0.0115 mol) was dissolved in DMF (35 ml), f llowed by additi n

of DhbtOH (2.07 g, 0.0127 mol) and 1-thyminylacetic acid (2.34 g, 0.0127 mol). Methylene chloride (35 ml) was add d and the mixture cooled to 0°C on an ice bath. After addition of DCC (2.85 g, 0.0138 mol), the mixture was stirred at 0°C for 2 h, followed by 1 h at room temperature. The precipitated DCU was removed by filtration, washed with methylene chloride (25 ml), and a further amount of methylene chloride (150 ml) was added to the filtrate. The organic phase was extracted with sodium hydrogen carbonate (1 volume saturated diluted with 1 volume water, 6 x 250 ml), potassium sulfate (1 volume saturated diluted with 4 volumes water, 3 x 250 ml), and saturated aqueous sodium chloride (1 x 250 ml), dried over magnesium sulfate, and evaporated to dryness, *in vacuo*. The solid residue was suspended in methylene chloride (35 ml) and stirred for 1h. The precipitated DCU was removed by filtration and washed with methylene chloride (25 ml). The filtrate was evaporated to dryness, *in vacuo*, and the residue purified by column chromatography on silica gel, eluting with a mixture of methanol and methylene chloride (gradient from 3-7% methanol in methylene chloride). This afforded the title compound as a white solid (3.05 g, 64%). M.p.. 76-79°C (decomp.). Anal. for $C_{18}H_{28}N_4O_7$, found (calc.) C: 52.03 (52.42) H: 6.90 (6.84) N: 13.21 (13.58). The compound showed satisfactory ^{1}H and ^{13}C-NMR spectra.

EXAMPLE 44

N-(N'-Boc-3'-aminopropyl)-N-[(1-thyminyl)acetyl]glycine.

N-(N'-Boc-3'-aminopropyl)-N-[(1-thyminyl)acetyl]glycine methyl ester (3.02 g, 0.00732 mol) was dissolved in methanol (25 ml) and stirred for 1.5 h with 2 M sodium hydroxide (25 ml). The methanol was removed by evaporation, *in vacuo*, and pH adjusted to 2 with 4 M hydrochloric acid at 0°C. The product was isolated as white crystals by filtration, washed with water (3 x 10 ml), and dried ver sicapent, *in vacuo*. Yield 2.19 g (75%). Anal. f r $C_{17}H_{26}N_4O_7$, H_2O, f und (calc.) C: 49.95 (49.03) H: 6.47 (6.29) N: 13.43 (13.45). The compound showed satisfactory ^{1}H and ^{13}C-NMR spectra.

EXAMPLE 45

3-(1-Thyminyl)-propan ic acid methyl ester.

Thymine (14.0 g, 0.11 mol) was suspended in methanol. Methyl acrylate (39.6 ml, 0.44 mol) was added, along with catalytic amounts of sodium hydroxide. The solution was refluxed in the dark for 45 h, evaporated to dryness, *in vacuo*, and the residue dissolved in methanol (8 ml) with heating. After cooling on an ice bath, the product was precipitated by addition of ether (20 ml), isolated by filtration, washed with ether (3 x 15 ml), and dried over sicapent, *in vacuo*. Yield 11.23 g (48%). M.p. 112-119°C. Anal. for $C_9H_{12}N_2O_4$, found (calc.) C: 51.14 (50.94) H: 5.78 (5.70) N: 11.52 (13.20). The compound showed satisfactory ^1H and ^{13}C-NMR spectra.

EXAMPLE 46

3-(1-Thyminyl)-propanoic acid.

3-(1-Thyminyl)-propanoic acid methyl ester (1.0 g, 0.0047 mol) was suspended in 2 M sodium hydroxide (15 ml), boiled for 10 min. The pH was adjusted to 0.3 with conc. hydrochloric acid. The solution was extracted with ethyl acetate (10 x 25 ml). The organic phase was extracted with saturated aqueous sodium chloride, dried over magnesium sulfate, and evaporated to dryness, *in vacuo*, to give the title compound as a white solid (0.66 g, 71%). M.p. 118-121°C. Anal. for $C_8H_{10}N_2O_4$, found (calc.) C: 48.38 (48.49) H: 5.09 (5.09) N: 13.93 (14.14). The compound showed satisfactory ^1H and ^{13}C-NMR spectra.

EXAMPLE 47

N-(N'-Boc-aminoethyl)-N-[(1-thyminyl)propanoyl]glycine ethyl ester.

N-(N'-Boc-aminoethyl)glycine ethyl ester (1.0 g, 0.0041 mol) was dissolved in DMF (12 ml). DhbtOH (0.73 g, 0.0045 mol) and 3-(1-thyminyl)-propanoic acid (0.89 g, 0.0045 m l) were added. Methylene chloride (12 ml) then was added and the mixture was cooled to 0°C on an ice bath. After addition f

DCC (1.01 g, 0.0049 mol), the mixture was stirr d at 0°C for 2 h, followed by 1 h at ro m temperature. The pr cipitated DCU was removed by filtration, washed with methylene chloride (25 ml), and a further amount of methylene chloride (50 ml) was added to the filtrate. The organic phase was extracted with sodium hydrogen carbonate (1 volume saturated diluted with 1 volume water, 6 x 100 ml), potassium sulfate (1 volume saturated diluted with 4 volumes water, 3 x 100 ml), and saturated aqueous sodium chloride (1 x 100 ml), dried over magnesium sulfate, and evaporated to dryness, *in vacuo*. Th solid residue was suspended in methylene chloride (15 ml), and stirred for 1h. The precipitated DCU was removed by filtration and washed with methylene chloride. The filtrat was evaporated to dryness, *in vacuo*, and the residue purified by column chromatography on silica gel, eluting with a mixtur of methanol and methylene chloride (gradient from 1 to 6% methanol in methylene chloride). This afforded the titl compound as a white solid (1.02 g, 59%). Anal. for $C_{19}H_{30}N_4O_7$, found (calc.) C: 53.15 (53.51) H: 6.90 (7.09) N: 12.76 (13.13). The compound showed satisfactory ^1H and ^{13}C-NMR spectra.

EXAMPLE 48

N-(N′-Boc-aminoethyl)-N-[(1-thyminyl)propanoyl]glycine .

N-(N′-Boc-aminoethyl)-N-[(1-thyminyl)propanoyl]glycin ethyl ester (0.83 g, 0.00195 mol) was dissolved in methanol (25 ml). Sodium hydroxide (25 ml; 2 M) was added. The solution was stirred for 1 h. The methanol was removed by evaporation, *in vacuo*, and the pH adjusted to 2 with 4 M hydrochloric acid at 0°C. The product was isolated by filtration, washed with ether (3 x 15 ml), and dried over sicapent, *in vacuo*. Yield 0.769 g, 99%). M.p. 213°C (decomp.).

EXAMPLE 49

M n -B c-ethylenediamine (2).

tert-Butyl-4-nitrophenyl carbonate (1) (10.0 g; 0.0418 mol) dissolved in DMF (50 ml) was added dropwise over a period of 30 min to a solution of ethylenediamine (27.9 ml; 0.418 mol) and DMF (50 ml) and stirred overnight. The mixture was evaporated to dryness, *in vacuo*, and the resulting oil dissolved in water (250 ml). After cooling to 0°C, pH was adjusted to 3.5 with 4 M hydrochloric acid. The solution then was filtered and extracted with chloroform (3x250 ml). The pH was adjusted to 12 at 0°C with 2 M sodium hydroxide, and the aqueous solution extracted with methylene chloride (3x300 ml). After treatment with sat. aqueous sodium chloride (250 ml), the methylene chloride solution was dried over magnesium sulfate. After filtration, the solution was evaporated t dryness, *in vacuo*, resulting in 4.22 g (63%) of the product (oil). ^{1}H-NMR (90 MHz; $CDCl_3$): δ1.44 (s, 9H); 2.87 (t, 2H); 3.1 (q, 2H); 5.62 (s, broad).

EXAMPLE 50

(N-Boc-aminoethyl)-β-alanine methyl ester, HCl.

Mono-Boc-ethylenediamine (2) (16.28 g; 0.102mol) was dissolved in acetonitrile (400 ml) and methyl acrylate (91.50 ml; 1.02 mol) was transferred to the mixture with acetonitrile (200 ml). The solution was refluxed overnight under nitrogen in the dark to avoid polymerization of methyl acrylate. After evaporation to dryness, *in vacuo*, a mixture of water and ether (200 + 200 ml) was added, and the solution was filtered and vigorously stirred. The aqueous phase was extracted one more time with ether and then freeze dried to yield a yellow solid. Recrystallization from ethyl acetate yielded 13.09 g (46%) of the title compound. M.p. 138-140°C. Anal. for $C_{11}H_{23}N_2O_4Cl$, found (calc.) C: 46.49 (46.72) H: 8.38 (8.20) N: 9.83 (9.91) Cl: 12.45 (12.54). ^{1}H-NMR (90 MHz; DMSO-d_6): δ 1.39 (s, 9H); 2.9 (m, 8H); 3.64 (s, 3H).

EXAMPLE 51

N-[(1-Thyminyl)acetyl]-N'-Boc-aminoethyl-β-alanine methyl ester.

(N-Boc-amino-ethyl)-β-alanine methyl ester, HCl (3) (2.0 g; 0.0071 mol) and 1-thyminylacetic acid pentafluorophenyl ester (5) (2.828 g; 0.00812 mol) were dissolved in DMF (50 ml). Triethyl amine (1.12 ml; 0.00812 mol) was added and the mixture stirred overnight. After addition of methylene chloride (200 ml) the organic phase was extracted with aqueous sodium hydrogen carbonate (3x250 ml), half-sat. aqueous potassium hydrogen sulfate (3x250 ml), and sat. aqueous sodium chloride (250 ml) and dried over magnesium sulfate. Filtration and evaporation to dryness, *in vacuo*, resulted in 2.9 g (99%) yield (oil). ^{1}H-NMR (250 MHz; $CDCl_3$): due to limited rotation around the secondary amide several of the signals were doubled; δ 1.43 (s, 9H); 1.88 (s, 3H); 2.63 (t, 1H); 2.74 (t, 1H); 3.25-3.55 (4xt, 8H); 3.65 (2xt, 2H); 3.66 (s, 1.5); 3.72 (s, 1.5); 4.61 (s, 1H); 4.72 (s, 2H); 5.59 (s, 0.5H); 5.96 (s, 0.5H); 7.11 (s, 1H); 10.33 (s, 1H).

EXAMPLE 52

N-[(1-Thyminyl)acetyl]-N'-Boc-aminoethyl-β-alanine.

N-[(1-Thyminyl)acetyl]-N'-Boc-aminoethyl-β-alanine methyl ester (3.0 g; 0.0073 mol) was dissolved in 2 M sodium hydroxide (30 ml), the pH adjusted to 2 at 0°C with 4 M hydrochloric acid, and the solution stirred for 2 h. The precipitate was isolated by filtration, washed three times with cold water, and dried over sicapent, *in vacuo*. Yield 2.23 g (77%). M.p. 170-176°C. Anal. for $C_{17}H_{26}N_4O_7$, H_2O, found (calc.) C: 49.49 (49.03) H: 6.31 (6.78) N: 13.84 (13.45). ^{1}H-NMR (90 MHz; DMSO-d_6): δ 1.38 (s, 9H); 1.76 (s, 3H); 2.44 and 3.29 (m, 8H); 4.55 (s, 2H); 7.3 (s, 1H); 11.23 (s, 1H). FAB-MS: 399 (M+1).

EXAMPLE 53

N-[(1-(N^4-Z)-cyt syl)acetyl]-N'-B c-aminoethyl-β-alani n methyl ester.

(N-Boc-amino-ethyl)-β-alanine methyl ester, HCl (3) (2.0 g; 0.0071 mol) and 1-(N-4-Z)-cytosylacetic acid pentafluorophenyl ester (5) (3.319 g; 0.0071 mol) were dissolved in DMF (50 ml). Triethyl amine (0.99 ml; 0.0071 mol) was added and the mixture stirred overnight. After addition of methylene chloride (200 ml), the organic phase was extracted with aqueous sodium hydrogen carbonate (3x250 ml), half-sat. aqueous potassium hydrogen sulfate (3x250 ml), and sat. aqueous sodium chloride (250 ml), and dried over magnesium sulfate. Filtration and evaporation to dryness, *in vacuo*, resulted in 3.36 g of solid compound which was recrystallized from methanol. Yield 2.42 g (64%). M.p. 158-161°C. Anal. for $C_{25}H_{33}N_5O_8$, found (calc.) C: 55.19 (56.49) H: 6.19 (6.26) N: 12.86 (13.18). ^{1}H-NMR (250 MHz; $CDCl_3$): due to limited rotation around the secondary amide several of the signals were doubled; δ 1.43 (s, 9H); 2.57 (t, 1H); 3.60-3.23 (m's, 6H); 3.60 (s, 1,5H); 3.66 (s, 1.5H); 4.80 (s, 1H); 4.88 (s, 1H); 5.20 (s, 2H); 7.80-7.25 (m's, 7H). FAB-MS: 532 (M+1).

EXAMPLE 54

N-[(1-(N^4-Z)-cytosyl)acetyl]-N'-Boc-aminoethyl-β-alanine.

N-[(1--(N-4-Z)-cytosyl)acetyl]-N'-Boc-aminoethyl-β-alanine methyl ester (0.621 g; 0.0012 mol) was dissolved in 2 M sodium hydroxide (8.5 ml) and stirred for 2h. Subsequently, pH was adjusted to 2 at 0°C with 4 M hydrochloric acid and the solution stirred for 2 h. The precipitate was isolated by filtration, washed three times with cold water, and dried over sicapent, *in vacuo*. Yield 0.326 g (54%). The white solid was recrystallized from 2-propanol and washed with petroleum ether. Mp.163°C (decomp.). Anal. f r $C_{24}H_{31}N_5O_8$, f und (calc.) C: 49.49 (49.03) H: 6.31 (6.78) N: 13.84 (13.45). ^{1}H-NMR (250 MHz; $CDCl_3$): due to limited rotati n around the secondary amide several of the

signals were doubled; δ 1.40 (s, 9H); 2.57 (t, 1H); 2.65 (t, 1H); 3.60-3.32 (m's, 6H); 4.85 (s, 1H); 4.98 (s, 1H); 5.21 (s, 2H); 5.71 (s, 1H, broad); 7.99-7.25 (m's, 7H). FAB-MS: 518 (M+1).

EXAMPLE 55

Example of a PNA-oligomer with a guanine residue

(a) Solid-Phase Synthesis of H-[Taeg]$_5$-[Gaeg]-[Taeg]$_4$-Lys-NH_2

The protected PNA was assembled onto a Boc-Lys(ClZ) modified MBHA resin with a substitution of approximately 0.15 mmol/g (determined by quantitative Ninhydrin reaction). Capping of uncoupled amino groups was only carried out before the incorporation of the BocGaeg-OH monomer.

(b) Stepwise Assembly of H-[Taeg]$_5$-[Gaeg]-[Taeg]$_4$-Lys-NH_2 (synthetic protocol)

Synthesis was initiated on 102 mg (dry weight) of preswollen (overnight in DCM) and neutralized Boc-Lys(ClZ)-MBHA resin. The steps performed were as follows: (1) Boc-deprotection with TFA/DCM (1:1, v/v), 1 x 2 min and 1 x 1/2 h, 3 ml; (2) washing with DCM, 4 x 20 sec, 3 ml; washing with DMF, 2 x 20 sec, 3 ml; washing with DCM, 2 x 20 sec, 3 ml, and drain for 30 sec; (3) neutralization with DIEA/DCM (1:19 v/v), 2 x 3 min, 3 ml; (4) washing with DCM, 4 x 20 sec, 3 ml, and drain for 1 min.; (5) addition of 4 equiv. diisopropyl carbodiimide (0.06 mmol; 9.7 μl) and 4 equiv. (0.06 mmol; 24 mg) BocTaeg-OH or (0.06 mmol; 30 mg) BocGaeg-OH dissolved in 0.6 ml DCM/DMF (1:1, v/v) (final concentration of monomer 0.1 M), the coupling reaction was allowed to proceed for 1/2 h shaking at room temperature; (6) drain for 20 sec; (7) washing with DMF, 2 x 20 sec and 1 x 2 min, 3 ml; washing with DCM 4 x 20 sec, 3 ml; (8) neutralization with DIEA/DCM (1:19 v/v), 2 x 3 min, 3 ml; (9) washing with DCM 4 x 20 sec, 3 ml, and drain for 1 min.; (10) qualitative Kaiser test; (11) blocking f unreacted amin groups by ac tylation with Ac_2O/pyridine/DCM (1:1:2, v/v), 1 x 1/2 h, 3 ml; and (12) washing with DCM, 4 x 20 sec, 2 x 2

min and 2 x 20 sec, 3 ml. Steps 1-12 were repeated until the desired sequence was obtained. All qualitative Kaiser tests were negative (straw-yellow colour with no coloration of the beads) indicating near 100% coupling yield. The PNA-oligomer was cleaved and purified by the normal procedure. FAB-MS: 2832.11 [M^*+1] (calc. 2832.15)

EXAMPLE 56

Solid-Phase Synthesis of H-Taeg-Aaeg-[Taeg]$_8$-Lys-NH_2.

(a) Stepwise Assembly of Boc-Taeg-A(Z)aeg-[Taeg]$_8$-Lys(ClZ)-MBHA Resin.

About 0.3 g of wet Boc-[Taeg]$_8$-Lys(ClZ)-MBHA resin was placed in a 3 ml SPPS reaction vessel. Boc-Taeg-A(Z)aeg-[Taeg]$_8$-Lys(ClZ)-MBHA resin was assembled by *in situ* DCC coupling (single) of the A(Z)aeg residue utilizing 0.19 M f BocA(Z)aeg-OH together with 0.15 M DCC in 2.5 ml 50% DMF/CH_2Cl_2 and a single coupling with 0.15 M BocTaeg-OPfp in neat CH_2Cl_2 ("Synthetic Protocol 5"). The synthesis was monitored by the quantitative ninhydrin reaction, which showed about 50% incorporation of A(Z)aeg and about 96% incorporation of Taeg.

(b) Cleavage, Purification, and Identification of H-Taeg-Aaeg-[Taeg]$_8$-Lys-NH_2.

The protected Boc-Taeg-A(Z)aeg-[Taeg]$_8$-Lys(ClZ)-BAH resin was treated as described in Example 40c to yield about 15.6 mg of crude material upon HF cleavage of 53.1 mg dry H-Taeg-A(Z)aeg-[Taeg]$_8$-Lys(ClZ)-BHA resin. The main peak at 14.4 min accounted for less than 50% of the total absorbance. A 0.5 mg portion of the crude product was purified to give approximately 0.1 mg of H-Taeg-Aaeg-[Taeg]$_8$-Lys-NH_2. For $(MH+)^+$ the calculated m/z value was 2816.16 and the measured m/z value was 2816.28.

(c) Synthetic Protocol 5

(1) Boc-deprotection with TFA/CH_2Cl_2 (1:1, v/v), 2.5 ml, 3 x 1 min and 1 x 30 min; (2) washing with CH_2Cl_2, 2.5 ml, 6 x 1 min; (3) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 2.5 ml, 3 x 2 min; (4) washing with CH_2Cl_2, 2.5 ml, 6 x 1 min, and drain for 1 min; (5) 2-5 mg sample f PNA-resin is taken out

and dried th roughly for a quantitative ninhydrin analysis to determine the substituti n; (6) addition of 0.47 mmol (0.25 g) BocA(Z)aeg-OH dissolved in 1.25 ml DMF followed by addition of 0.47 mmol (0.1 g) DCC in 1.25 ml CH_2Cl_2 or 0.36 mmol (0.20 g) BocTaeg-OPfp in 2.5 ml CH_2Cl_2; the coupling reaction was allowed to proceed for a total of 20-24 hrs shaking; (7) washing with DMF, 2.5 ml, 1 x 2 min; (8) washing with CH_2Cl_2, 2.5 ml, 4 x 1 min; (9) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 2.5 ml, 2 x 2 min; (10) washing with CH_2Cl_2, 2.5 ml, 6 x 1 min; (11) 2-5 mg sample of protected PNA-resin is taken out and dried thoroughly for a quantitative ninhydrin analysis to determine the extent of coupling; (12) blocking of unreacted amino groups by acetylation with a 25 ml mixture of acetic anhydride/pyridine/CH_2Cl_2 (1:1:2, v/v/v) for 2 h (except after the last cycle); and (13) washing with CH_2Cl_2, 2.5 ml, 6 x 1 min; (14) 2 x 2-5 mg samples of protected PNA-resin are taken out, neutralized with DIEA/CH_2Cl_2 (1: 19, v/v) and washed with CH_2Cl_2 for ninhydrin analyses.

EXAMPLE 57

Solid-Phase Synthesis of H-[Taeg]$_2$-Aaeg-[Taeg]$_5$-Lys-NH$_2$.

(a) Stepwise Assembly of Boc-[Taeg]$_2$-A(Z)aeg-[Taeg]$_5$-Lys(ClZ)-MBHA Resin.

About 0.5 g of wet Boc-[Taeg]$_5$-Lys(ClZ)-MBHA resin was placed in a 5 ml SPPS reaction vessel. Boc-[Taeg]$_2$-A(Z)aeg-[Taeg]$_5$-Lys(ClZ)-MBHA resin was assembled by *in situ* DCC coupling of both the A(Z)aeg and the Taeg residues utilising 0.15 M to 0.2 M of protected PNA monomer (free acid) together with an equivalent amount of DCC in 2 ml neat CH_2Cl_2 ("Synthetic Protocol 6"). The synthesis was monitored by the quantitative ninhydrin reaction which showed a total of about 82% incorporation of A(Z)aeg after coupling three times (the first coupling gave about 50% incorp ration; a fourth HOBt-mediated coupling in 50% DMF/CH2Cl2 did not increas th total coupling yield significantly) and quantitative inc rporation (single couplings) f the Taeg residues.

(b) Cleavage, Purificati n, and Identificati n f H-[Taeg]$_2$-Aaeg-[Ta g]$_5$-Lys-NH_2.

The protected Boc-[Taeg]$_2$-A(Z)aeg-[Taeg]$_5$-Lys(ClZ)-BHA resin was treated as described in Example 40c to yield about 16.2 mg of crude material upon HF cleavage of 102.5 mg dry H-[Taeg]$_2$-A(Z)aeg-[Taeg]$_5$-Lys(ClZ)-BHA resin. A small portion of the crude product was purified. For $(MH+)^+$, the calculated m/z value was 2050.85 and the measured m/z value was 2050.90

(c) Synthetic Protocol 6

(1) Boc-deprotection with TFA/CH_2Cl_2 (1:1, v/v), 2 ml, 3 x 1 min and 1 x 30 min; (2) washing with CH_2Cl_2, 2 ml, 6 x 1 min; (3) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 2 ml, 3 x 2 min; (4) washing with CH_2Cl_2, 2 ml, 6 x 1 min, and drain for 1 min; (5) 2-5 mg sample of PNA-resin was taken out and dried thoroughly for a quantitative ninhydrin analysis to determine the substitution; (6) addition of 0.44 mmol (0.23 g) BocA(Z)aeg-OH dissolved in 1.5 ml CH_2Cl_2 followed by addition of 0.44 mmol (0.09 g) DCC in 0.5 ml CH_2Cl_2 or 0.33 mmol (0.13 g) BocTaeg-OH in 1.5 ml CH_2Cl_2 followed by addition of 0.33 mmol (0.07 g) DCC in 0.5 ml CH_2Cl_2; the coupling reaction was allowed to proceed for a total of 20-24 hrs with shaking; (7) washing with DMF, 2 ml, 1 x 2 min; (8) washing with CH_2Cl_2, 2 ml, 4 x 1 min; (9) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 2 ml, 2 x 2 min; (10) washing with CH_2Cl_2, 2 ml, 6 x 1 min; (11) 2-5 mg sample of protected PNA-resin is taken out and dried thoroughly for a quantitative ninhydrin analysis to determine the extent of coupling; (12) blocking of unreacted amino groups by acetylation with a 25 ml mixture of acetic anhydride/pyridine/CH_2Cl_2 (1:1:2, v/v/v) for 2 h (except after the last cycle); (13) washing with CH_2Cl_2, 2 ml, 6 x 1 min; and (14) 2 x 2-5 mg samples of protected PNA-resin were taken out, neutralized with DIEA/CH_2Cl_2 (1: 19, v/v) and washed with CH_2Cl_2 for ninhydrin analyses.

EXAMPLE 58

The PNA-olig mer H-T4C2TCT-LysNH_2 was prepared as described in Example 93. Hybridization experiments with this sequence should resolve the issue of orientation, since it is truly asymmetrical. Such experiments should also resolve the issues of pH-dependency of the Tm, and the stoichiometry of complexes formed.

Hybridization experiments with the PNA-oligomer H-T_4C_2TCTC-LysNH_2 were performed as follows:

Row	Hybridized With	pH	Tm	S
1	5'-$(dA)_4(dG)_2$(dA)(dG)(dA)(dG)	7.2	55.5	2:1
2	5'-$(dA)_4(dG)_2$(dA)(dG)(dA)(dG)	9.0	26.0	2:1
3	5'-$(dA)_4(dG)_2$(dA)(dG)(dA)(dG)	5.0	88.5	2:1
4	5'-(dG)(dA)(dG)(dA)$(dG)_2(dA)_4$	7.2	38.0	2:1
5	5'-(dG)(dA)(dG)(dA)$(dG)_2(dA)_4$	9.0	31.5	-
6	5'-(dG)(dA)(dG)(dA)$(dG)_2(dA)_4$	5.0	52.5	-
7	5'-$(dA)_4$(dG)(dT)(dA)(dG)(dA)(dG)	7.2	39.0	-
8	5'-$(dA)_4$(dG)(dT)(dA)(dG)(dA)(dG)	9.0	<20	-
9	5'-$(dA)_4$(dG)(dT)(dA)(dG)(dA)(dG)	5.0	51.5	-
10	5'-$(dA)_4(dG)_2$(dT)(dG)(dA)(dG)	7.2	31.5	-
11	5'-$(dA)_4(dG)_2$(dT)(dG)(dA)(dG)	5.0	50.5	-
12	5'-(dG)(dA)(dG)(dA)dT)(dG)$(dA)_4$	7.2	24.5	-
13	5'-(dG)(dA)(dG)(dA)dT)(dG)$(dA)_4$	9.0	<20	-
14	5'-(dG)(dA)(dG)(dA)dT)(dG)$(dA)_4$	5.0	57.0	-
15	5'-(dG)(dA)(dG)(dT)$(dG)_2(dA)_4$	7.2	25.0	-
16	5'-(dG)(dA)(dG)(dT)$(dG)_2(dA)_4$	5.0	39.5	-
			52.0	

S = stoichiometry determined by UV-mixing curves
\- = not determined

These results show that a truly mixed sequence gave rise t well defined m lting curves. The PNA-oligomers can actually bind in both orientations (compare r w 1 and 4), although there is pref rence for the N-terminal/5'-

orientation. Introducing a single mismatch opp sit either T or C caus d a lowering of T_m by m re than 16°C at pH 7.2; at pH 5.0 the T_m-value was lowered more than 27°C. This shows that there is a very high degree a sequence-selectivity which should be a general feature for all PNA C/T sequences.

As indicated above, there is a very strong pH-dependency for the T_m-value, indicating that Heogsteen basepairing is important for the formation of hybrids. Therefore, it is not surprising that the stoichiometry was found to be 2:1.

The lack of symmetry in the sequence and the very large lowering of T_m when mismatches are present show that th Watson-Crick strand and the Hoogsteen strand are parallel when bound to complementary DNA. This is true for both of the orientations, *i.e.*, 5'/N-terminal and 3'/N-terminal.

EXAMPLE 59

The results of hybridization experiments with H-T_5GT_4-$LysNH_2$ to were performed as follows:

Row	Deoxyoligonucleotide	Tm
1	5'-(dA)5(dA)(dA)4-3'	55.0
2	5'-(dA)5(dG)(dA)4-3'	47.0
3	5'-(dA)5(dG)(dA)4-3'	56.5
4	5'-(dA)5(dT)(dA)4-3'	46.5
5	5'-(dA)4(dG)(dA)5-3'	48.5
6	5'-(dA)4(dC)(dA)5-3'	55.5
7	5'-(dA)4(dT)(dA)5-3'	47.0

As shown by comparing rows 1, 3, and 6 with rows 2, 4, 5, and 7, G can in this mode discriminate between C/A and G/T in the DNA-strand, *i.e.*, sequence discrimination is observed. The complex in row 3 was furthermore determined to be 2 PNA: 1 DNA complex by UV-mixing curves.

EXAMPLE 60

The mass s f s me synthesiz d PNA-oligomers, as determined by FAB mass spectrometry, are as follows:

SEQUENCE	CALC.	FOUND
H-T_4C_2TCTC-LysNH_2	2747.15	2746.78
H-T_5GT_4-LysNH_2	2832.15	2832.11
H-T_7-LysNH_2	2008.84	2540.84
H-T_9-LysNH_2	2541.04	2540.84
H-T_{10}-LysNH_2	2807.14	2806.69
H-T_2CT_5-LysNH_2	2259.94	2259.18
H-T_3(L-alaT)T_4-LysNH_2	2287.95	2288.60
H-T_4(Ac)T_5-LysNH_2	2683.12	2683.09

EXAMPLE 61

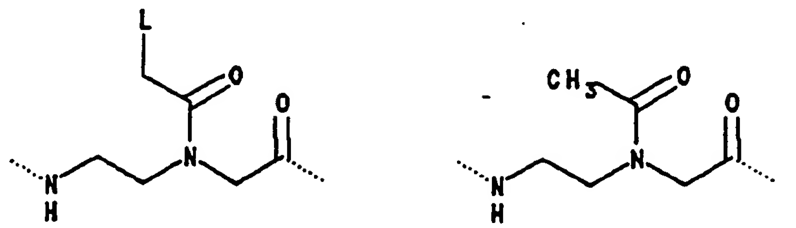
Hybridization data for a PNA-oligomer with a single unit with an extended backbone (the β-alanine modification) is as follows:

PNA	DNA	T_m
H-T_{10}-LysnH_2	$(dA)_{10}$	73°C
H-T_4(βT)T_5-LysNH_2	$(dA)_{10}$	57°C
H-T_4(βT)T_5-LysNH_2	$(dA)_4(dG)(dA)_5$	47°C
H-T_4(βT)T_5-LysNH_2	$(dA)_4(dT)(dA)_5$	49°C
H-T_4(βT)T_5-LysNH_2	$(dA)_4(dT)(dA)_5$	47°C

Although the melting temperature decreases, the data demonstrates that base specific recognition is retained.

EXAMPLE 62

An example with a "no base" substitution.



i.e. L=H

PNA	DNA	T_m
H-T_{10}-LysNH$_2$	$(dA)_{10}$	73°C
H-T_4(Ac)T_5-LysNH$_2$	$(dA)_{10}$	49°C
H-T_4(Ac)T_5-LysNH$_2$	$(dA)_4(dG)(dA)^5$	37°C
H-T_4(Ac)T_5-LysNH$_2$	$(dA)_4(dC)(dA)^5$	41°C
H-T_4(Ac)T_5-LysNH$_2$	$(dA)_4(dT)(dA)^5$	41°C
H-T_4(Ac)T_5-LysNH$_2$	$(dA)_5(dG)(dA)^4$	36°C
H-T_4(Ac)T_5-LysNH$_2$	$(dA)_5(dC)(dA)^4$	40°C
H-T_4(Ac)T_5-LysNH$_2$	$(dA)_5(dT)(dA)^4$	40°C

EXAMPLE 63

Iodination Procedure

A 5 µg portion of Tyr-PNA-T_{10}-Lys-NH$_2$ is dissolved in 40 µl 100 mM Na-phosphate, pH 7.0, and 1 mCi Na^{125}I and 2 µl chloramine-T (50 mM in CH_3CN) are added. The solution is left at 20°C for 10 min and then passed through a 0.5 + 5 cm Sephadex G10 column. The first 2 fractions (100 µl each) containing radioactivity are collected and purified by HPLC: reversed phase C-18 using a 0-60% CH_3CN gradient in 0.1% CF_3COOH in H_2O. The ^{125}I-PNA elutes right after the PNA peak. The solvent is removed under reduced pressure.

EXAMPLE 64

Binding of PNAs-T_{10}/T_9C/T_8C_2 t d uble stranded DNA targ ts A_{10}/A_9G/A_8G_2 (Figure 20).

A mixture of 200 cps ^{32}P-labeled *Eco*RI-*Pvu*II fragment (the large fragment labeled at the 3'-end of the *Eco*RI site) of the indicated plasmid, 0.5 μg carrier calf thymus DNA, and 300 ng PNA in 100 μl buffer (200 mM NaCl, 50 mM Na-acetate, pH 4.5, 1 mM $ZnSO_4$) was incubated at 37°C for 120 min. A 50 unit portion of nuclease S_1 was added and incubated at 20°C for 5 min. The reaction was stopped by addition of 3 μl 0.5 M EDTA and the DNA was precipitated by addition of 250 μl 2% potassium acetate in ethanol. The DNA was analyzed by electrophoresis in 10% polyacrylamide sequencing gels and the radiolabeled DNA bands visualized by autoradiography.

The target plasmids were prepared by cloning of the appropriate oligonucleotides into pUC19. Target A_{10}: oligonucleotides GATCCA_{10}G & GATCCT_{10}G cloned into the *Bam*HI site (plasmid designated pT10). Target A_5GA_4: oligonucleotides TCGACT_4CT_5G & TCGACA_5GA_4G cloned into the *Sal*I site (plasmid pT9C). Target $A_2GA_2GA_4$: oligonucleotides GA_2GA_2GA_4TGCA & GT_4CT_2CT_2CTGCA into the *Pst*I site (plasmid pT8C2). The positions of the targets in the gel are indicated by bars to the left. A/G is an A+G sequence ladder of target P10.

EXAMPLE 65

Inhibition of restriction enzyme cleavage by PNA (Figure 23).

A 2 μg portion of plasmid pT10 was mixed with the indicated amount of PNA-T_{10} in 20 μl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4) and incubated at 37°C for 120 min. 2 μl 10 × buffer (10 mM Tris-HCl, pH 7.5, 10 mM, $MgCl_2$, 50 mM NaCl, 1 mM DTT). *Pvu*II (2 units) and *Bam*HI (2 units) were added and the incubation was continued for 60 min. The DNA was analyzed by gel electrophoresis in 5% polyacrylamide and the DNA was visualized by ethidium bromide staining.

EXAMPLE 66

Kinetics f PNA-T_{10} - dsDNA strand displacement c mplex formation (Figure 21).

A mixture of 200 cps ^{32}P-labeled *Eco*RI-*Pvu*II fragment of pT10 (the large fragment labeled at the 3'-end of the *Eco*RI site), 0.5 μg carrier calf thymus DNA, and 300 ng of PNA-T_{10}-$LysNH_2$ in 100 μl buffer (200 mM NaCl, 50 mM Na-acetate, pH 4.5, 1 mM $ZnSO_4$) were incubated at 37°C. At the times indicated, 50 U of S_1 nuclease was added to each of 7 samples and incubation was continued for 5 min at 20°C. The DNA was then precipitated by addition of 250 μl 2% K-acetate in ethanol and analyzed by electrophoresis in a 10% polyacrylamide sequencing gel. The amount of strand displacement complex was calculated from the intensity of the S_1-cleavage at the target sequence, as measured by densitometric scanning of autoradiographs.

EXAMPLE 67

Stability of PNA-dsDNA complexes (Figure 22).

A mixture of 200 cps ^{32}P-pT10 fragment, 0.5 μg calf thymus DNA and 300 ng of the desired PNA (either T_{10}-$LysNH_2$, T_8-$LysNH_2$ or T_6-$LysNH_2$) was incubated in 100 μl 200 mM NaCl, 50 mM Na-acetate, pH 4.5, 1 mM $ZnSO_4$ for 60 min at 37°C. A 2 μg portion of oligonucleotide GATCCA_{10}G was added and each sample was heated for 10 min at the temperature indicated, cooled in ice for 10 min and warmed to 20°C. A 50 U portion of S_1 nuclease was added and the samples treated and analyzed and the results quantified.

EXAMPLE 68

Inhibition of Transcription by PNA

A mixture of 100 ng plasmid DNA (cleaved with restriction enzyme *Pvu*II (see below) and 100 ng of PNA in 15 μl 10 mM Tris-HCl, 1 mM EDTA, pH 7.4 was incubated at 37°C for 60 min. Subs quently, 4 μl 5 × c ncentrated buffer (0.2 M Tris-HCl (pH 8.0), 40 mM $MgCl_2$, 10 mM spermidine, 125 mM NaCl) were mixed with 1 μl NTP-mix (10 mM ATP, 10 mM CTP, 10 mM GTP,

1 mM UTP, 0.1 μCi/μl ^{32}P-UTP, 5 mM DTT, 2 μg/ml tRNA, 1 μg/ml heparin) and 3 units RNA polymeras . Incubation was continued for 10 min at 37°C. The RNA was then precipitated by addition of 60 μl 2% postassium acetate in 96% ethanol at -20°C and analyzed by electrophoresis in 8% polyacrylamide sequencing gels. RNA transcripts were visualized by autoradiography. The following plasmids were used: pT8C2-KS/pA8G2-KS: oligonucleotides $GA_2GA_2GA_4GTGAC$ & $GT_4CT_2CT_2CTGCA$ cloned into the *PstI* site of pBluescript-KS$^+$; pT10-KS/pA10-KS (both orientations of the insert were obtained). pT10UV5: oligonucleotides $GATCCA_{10}G$ & $GATCCT_{10}G$ cloned into the *BamHI* site of a pUC18 derivative in which the lac UV5 *E.coli* promoter had been cloned into the *EcoRI* site (Jeppesen, *et al.*, *Nucleic Acids Res.*, 1988, *16*, 9545).

Using T_3-RNA polymerase, transcription elongation arrest was obtained with PNA-T_8C_2-LysNH$_2$ and the pA8G2-KS plasmid having the PNA recognition sequence on the template strand, but not with pT8C2-KS having the PNA recognition sequence on the non-template strand. Similar results were obtained with PNA-T10-LysNH$_2$ and the plasmids pA10-KS and pT10-KS. (*see*, Figure 25) Using *E.coli* RNA polymerase and the pT10UV5 plasmid (A_{10}-sequence on the template strand) transcription elongation arrest was obtained with PNA-T_{10}-LysNH$_2$.

EXAMPLE 69

Biological stability of PNA

A mixture of PNA-T_5 (10 μg) and a control, "normal" peptide (10 μg) in 40 μl 50 mM Tris-HCl, pH 7.4 was treated with varying amounts of peptidase from porcine intestinal mucosa or protease from Streptomyces caespitosus for 10 min at 37°C. The amount of PNA and peptide was determined by HPLC analysis (reversed phase C-18 column: 0-60% acetonitrile, 0.1% trifluoroac tic acid).

At peptidase/protease concentrations where complet degradation f the peptide was observed (no HPLC peak) the PNA was still intact.

EXAMPLE 70

Inhibiti n f ene Expressi n

A preferred assay to test the ability of peptide nucleic acids to inhibit expression of the E2 mRNA of papillomavirus is based on the well-documented transactivation properties of E2. Spalholtz, *et al.*, *J. Virol.*, 1987, *61*, 2128-2137. A reporter plasmid (E2RECAT) was constructed t contain the E2 responsive element, which functions as an E2 dependent enhancer. E2RECAT also contains the SV40 early promoter, an early polyadenylation signal, and the chloramphenicol acetyl transferase gene (CAT). Within the context of this plasmid, CAT expression is dependent upon expression of E2. The dependence of CAT expression on the presence of E2 has been tested by transfection of this plasmid into C127 cells transformed by BPV-1, uninfected C127 cells and C127 cells cotransfected with E2RECAT and an E2 expression vector.

A. Inhibition of BPV-1 E2 Expression

BPV-1 transformed C127 cells are plated in 12 well plates. Twenty four hours prior to transfection with E2RE1, cells are pretreated by addition of antisense PNAs to the growth medium at final concentrations of 5, 15 and 30 mM. The next day cells are transfected with 10 μg of E2RE1CAT by calcium phosphate precipitation. Ten micrograms of E2RE1CAT and 10 μg of carrier DNA (PUC 19) are mixed with 62 μl of 2 M $CaCl_2$ in a final volume of 250 μl of H_20, followed by addition of 250 μl of 2X HBSP (1.5 mM Na_2PO_2. 10 mM KCl, 280 mM NaCl, 12 mM glucose and 50 mM HEPES, pH 7.0) and incubated at room temperature for 30 minutes. One hundred microliters of this solution is added to each test well and allowed to incubate for 4 hours at 37°C. After incubation, cells are glycerol shocked for 1 minute at room temperature with 15% glycerol in 0.75 mM Na_2PO_2, 5 mM KCl, 140 mM NaCl, 6 mM glucose and 25 mM HEPES, pH 7.0. After shocking, cells are washed 2 times with serum free DMEM and refed with DMEM containing 10% fetal b vin serum and antisense oligonucle tide at the original concentration. Forty eight

hours after transfection c lls are harvested and assayed for CAT activity.

For determination of CAT activity, cells are washed 2 times with phosphate buffered saline and collected by scraping. Cells are resuspended in 100 µl of 250 mM Tris-HCl, pH 8.0 and disrupted by freeze-thawing 3 times. Twenty four microliters of cell extract is used for each assay. For each assay the following are mixed together in an 1.5 ml Eppendorf tube and incubated at 37°C for one hour: 25 µl of cell extract, 5 µl of 4 mM acetyl coenzyme A, 18 µl H_2O and 1 µl ^{14}C-chloramphenicol, 40-60 mCi/mM. After incubation, chloramphenicol (acetylated and nonacetylated forms) is extracted with ethyl acetate and evaporated to dryness. Samples are resuspended in 25 µl of ethyl acetate, spotted onto a TLC plate and chromatographed in chloroform:methanol (19:1). Chromatographs are analyzed by autoradiography. Spots corresponding to acetylated and nonacetylated ^{14}C-chloramphenicol are excised from the TLC plate and counted by liquid scintillation for quantitation of CAT activity. Peptide nucleic acids that depress CAT activity in a dose dependent fashion are considered positives.

B. Inhibition of HPV E2 Expression

The assay for inhibition of human papillomavirus (HPV) E2 by peptide nucleic acids is essentially the same as that for BPV-1 E2. For HPV assays appropriate HPVs are co-transfected into either CV-1 or A431 cells with PSV2NEO using the calcium phosphate method described above. Cells which take up DNA are selected for by culturing in media containing the antibiotic G418. G418-resistant cells are then analyzed for HPV DNA and RNA. Cells expressing E2 are used as target cells for antisense studies. For each PNA, cells are pretreated as above, transfected with E2RE1CAT, and analyzed for CAT activity as above. Peptide nucleic acids are considered to have a positive effect if they can depr ss CAT activity in a dose dependent fashi n.

EXAMPLE 71

Synthesis f PNA 15-mer C ntaining Four Naturally ccurring Nucleobases; H-[Taeg]-[Aaeg]-[Gaeg]-[Taeg]-[Taeg]-[Aaeg]-[Taeg]-[Caeg]-[Taeg]-[Caeg]-[Taeg]-[Aaeg]-[Taeg]-[Caeg]-[Taeg]-LYS-NH2.

The protected PNA was assembled onto a Boc-Lys(ClZ) modified MBHA resin with a substitution of approximately 0.145 mmol/g. Capping of uncoupled amino groups was only carried out before the incorporation of the BocGaeg-OH monomer.

Synthesis was initiated on 100 mg (dry weight) of neutralised Boc-Lys(ClA)-MBHA resin that had been preswollen overnight in DCM. The incorporation of the monomers followed the protocol of Example 32, except at step 5 for the incorporation of the BocAaeg-OH monomer. Step 5 for the present synthesis involved addition of 4 equiv. diisopropyl carbodiimide (0.06 ml; 9.7 μl) and 4 equiv. BocAaeg-OH (0.06 mmol; 32 mg) dissolved in 0.6 ml DCM/DMF (1:1, v/v) (final concentration of monomer 0.1M). The coupling reaction was allowed to proceed for 1 x 15 min and 1 x 60 min. (recoupling).

All qualitative Kaiser tests were negative (straw-yellow color with no coloration of the beads). The PNA-oligomer was cleaved and purified by the standard procedure. FAB-MS average mass found(calc.) (M+H) 4145.1 (4146.1).

EXAMPLE 72

Hybridization of H-TAGTTATCTCTATCT-LysNH$_2$

DNA -target	pH	Tm
5′----3′	5	60.5
5′----3′	7.2	43.0
5′----3′	9	38.5
3′----5′	5	64.5/49.0
3′----5′	7.2	53.5
3′----5′	9	51.5

Th fact that there is alm st n loss in Tm in going from pH 7.2 to 9.0 indicates that Ho gst n bas pairing is not involved. The increase in Tm in going from 7.2 to 5 is large for the parallel orientation and is probably due to the formation of a 2:1 complex. It is believed that the most favorable orientation in the Watson-Crick binding motif is the 3'/N-orientation and that in the Hoogsteen motif the 5'/N-orientation is the most stable. Thus, it may be the case that the most stable complex is with the two PNA's strands anti parallel.

There is apparently a very strong preference for a parallel orientation of the Hoogsteen strand. This seems to explain why even at pH 9 a 2:1 complex is seen with the 5'/N-orientation. Furthermore, it explains the small loss in going from pH 7.2 to 9 in the 3'/N, as this is probably a 1:1 complex.

EXAMPLE 73

Solid-Phase Synthesis of H-[Taeg]$_2$-Aaeg-Taeg-Caeg-Aaeg-Taeg-Caeg-Taeg-Caeg-Lys-NH2.

(a) Stepwise Assembly of Boc-[Taeg]2-A(Z)aeg-Taeg-C(Z)aeg-A(Z)aeg-Taeg-C(Z)a eg-Taeg-C(Z)aeg-Lys(ClZ)-MBHA Resin.

About 1 g of wet Boc-Lys(ClZ)-MBHA (0.28 mmol Lys/g) resin was placed in a 5 ml SPPS reaction vessel. Boc-[Taeg]2-A(Z)aeg-Taeg-C(Z)aeg-A(Z)aeg-Taeg-C(Z)aeg-Taeg-C(Z)aeg-Lys(ClZ)-MBHA resin was assembled by *in situ* DCC coupling of the five first residues utilizing 0.16 M of BocC[Z]-OH, BocTaeg-OH or BocA(Z)aeg-OH, together with 0.16 M DCC in 2.0 ml 50% DMF/CH2Cl2 ("Synthetic Protocol 9") and by analogous *in situ* DIC coupling of the five last residues ("Synthetic Protocol 10") . Each coupling reaction was allowed to proceed for a total of 20-24 hrs with shaking. The synthesis was monitored by the ninhydrin reaction, which showed nearly quantitative incorp rati n of all residues except of the first A(Z)aeg residu , which had to be coupled twice. The total

coupling yield was about 96% (first c upling, about 89% efficiency).

(b) Cleavage, Purification, and Identification of H-[Taeg]2-Aaeg-Taeg-Caeg-Aaeg-Taeg-Caeg-Taeg-Caeg-Lys-NH2.

The protected Boc-[Taeg]2-A(Z)aeg-Taeg-C(Z)aeg-A(Z)aeg-Taeg-C(Z)aeg-Taeg-C(Z)aeg-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 53.4 mg of crude material upon HF cleavage of 166.1 mg dry Boc-[Taeg]2-A(Z)aeg-Taeg-C(Z)aeg-A(Z)aeg-Taeg-C(Z)aeg-Taeg-C(Z)aeg-Lys(ClZ)-MBHA resin. The crude product (53.4 mg) was purified to give 18.3 mg of H-[Taeg]2-Aaeg-Taeg-Caeg-Aaeg-Taeg-Caeg-Taeg-Caeg-L ys-NH2. For (M+H)+, the calculated m/z value = 2780.17 and th measured m/z value = 2780.07.

EXAMPLE 74

Hybridization properties of H-TTA TCA TCT C-Lys-NH_2.

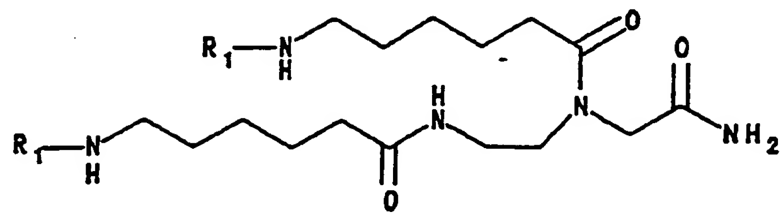
The title compound hybridized with the following oligonucleotides:

Oligodeoxynucleotide	pH	Tm(°C)
5'-AAT AGT AGT G-3	5	31.5†
5'-ATT AGT AGT G-3'	7.2	28.5†
5'-AAT AGT AGT G-3"	9	28.0†
5'-GTG ATG ATA A-3'	7.2	30.5
5'-GTG ATG ATA A-3'	9	28.0

†Low hypochromicity

EXAMPLE 75

Synthesis of a PNA With Two Parallel Strings Tied Together



A 375 mg portion of MBHA resin (loading 0.6 mmol/g) was allowed to swell over night in dichloromethane (DCM). After an hour in DMF/DCM, the resin was neutralized by washing 2 times with 5% diisopropylethylamine in DCM (2 min.), followed by washing with DCM (2ml; 6 x 1 min.) N,N'-di-Boc-aminoethyl glycine (41,9 mg; 0,132 mmol) disolved in 2 ml DMF was added to the resin, followed by DCC (64,9 mg; 0,315 mmol) dissolved in 1 ml of DCM. After 2.5 hours, the resin was washed with DMF 3 times (1 min.) and once with DCM (1 min.). The unreacted amino groups were then capped by treatment with acetic anhydride/DCM/pyridine (1 ml\2 ml\2 ml) for 72 hours. After washing with DCM (2 ml; 4 x 1 min), a Kaiser test showed no amino groups were present. The resin was deprotected and washed as described above. This was followed by reaction with 6-(Bocamino)-hexanoic acid DHBT ester (255.8 mg; 67 mmol) dissolved in DMF/DCM 1:1 (4 ml) overnight. After washing and neutraliation, a Kaiser test and an isatin test were performed. Both were negative. After capping, the elongenation of the PNA-chains was performed according to standard procedures for DCC couplings. All Kaiser tests performed after the coupling reactions were negative (Yellow). Qualitative Kaiser tests were done after deprotection of PNA units number 1, 2, 4, and 6. Each test was blue. The PNA oligomers were cleaved and purified by standard procedures.

The am unt f mon mer and DCC used f r each c upling was as f llows (total v lume 4.5 ml):

Coupling	Monomer(T)	DCC
1.	173 mg	95 mg
2.	176 mg	101 mg
3.	174 mg	97 mg
4.	174 mg	103 mg
5.	178 mg	97 mg
6.	173 mg	99 mg
7.	174 mg	95 mg
8.	175 mg	96 mg

For the PNA having the Structure (70) where $R_{70} = T_6$, there was 24.5 mg of crude product, which resulted in 6.9 mg. after purification. For the PNA where $R_1 = T_8$, there was 28.8 mg of crude product, which resulted in 2.8 mg. after purification. The products had a high tendency of aggregation, as indicated by a complex HPLC chromatogram after a few hours at room temperature in concentration above 1 mg/ml. The PNA-$(T_6)_2$ and PNA-$(T_8)_2$ were hybridised to $(dA)_6$ and $(dA)_8$, respectively, with recorded Tm of 42°C and 59°C, respectively.

EXAMPLE 76

Solid-Phase Synthesis of H-[Taeg]$_5$-Lys(ClZ)-MBHA Resin

The PNA oligomer was assembled onto 500 mg (dry weight) of MBHA resin that had been preswollen overnight in DCM. The resin was initially substituted with approximately 0.15 mmol/g Boc-Lys(ClZ) as determined by quantitative ninhydrin reaction. The stepwise synthesis of the oligomer followed the synthetic protocol described in Example 32 employing 0.077 g (0.2 mmol) B cTaeg-OH and 31.3 µl (0.2 mmol) diisopropyl carbodiimide in 2.0 ml 50% DMF/CH_2Cl_2 in each c upling. Capping of uncoupled amino groups was carri d out before deprotection in ach step. All qualitative Kaiser tests were negative indicating near 100% coupling yield.

EXAMPLE 77

Solid-Phase Synthesis of H-[Taeg]$_4$-[apgT]-[Taeg]$_5$Lys-NH$_2$

Synthesis was initiated on approximately 1/4 of the wet H-[Taeg]5-Lys(ClZ)-MBHA resin from Example 76. *In situ* diisopropyl carbodiimide (DIC) couplings of both Boc-(apgT)-OH and BocTaeg-OH were carried out in 1.2 ml 50% DMF/CH_2Cl_2 using 0.048 g (0.12 mmol) and 0.046 g (0.12 mmol) monomer, respectively, and 18.7 μl (0.12 mmol) diisopropyl carbodiimide in each coupling. All qualitative Kaiser tests were negative, indicating near 100% coupling yield. The PNA oligomer was cleaved and purified by standard procedures. For (M+H)+, the calculated m/z value was 2820.15 and the measured m/z value was 2820.92.

EXAMPLE 78

Solid-Phase Synthesis of H-[Taeg]$_4$-[proT]-[Taeg]$_5$-Lys-NH$_2$

Synthesis was initiated on approximately 1/4 of the wet H-[Taeg]$_5$-Lys(ClZ)-MBHA resin from Example 76. *In situ* diisopropyl carbodiimide couplings of BocTaeg-OH were carried out in 1.2 ml 50% DMF/CH_2Cl_2 using 0.046 g (0.12 mmol) monomer and 18.7 μl (0.12 mmol) diisopropyl carbodiimide in each coupling. Due to solubility problems, Boc-(proT)-OH 0.048 g (0.12 mmol) was suspended in 2.5 ml 50% DMF/DMSO prior to coupling, the suspension filtered, and approximately 2 ml of the filtrate used in the overnight coupling. All qualitative Kaiser tests were negative, indicating near 100% coupling yield. The PNA oligomer was cleaved and purified by standard procedures.

EXAMPLE 79

Hybridization pr perties f H-[Taeg]$_4$-[proT]-[Taeg]$_5$Lys-NH_2

Oligodeoxynucleotide	Tm(°C)
5'-AAA AAA AAA A	53.5
5-'AAA AGA AAA A	44.0
5'-AAA AAG AAA A	43.5
5'-AAA ACA AAA A	46.5
5'-AAA AAC AAA A	46.5
5'-AAA ATA AAA A	46.5
5'-AAA AAT AAA A	46.0

EXAMPLE 80

Solid-Phase Synthesis of H-[Taeg]$_4$-[bC]-[Taeg]$_5$-Lys-NH_2

The PNA oligomer was assembled onto 100 mg (dry weight) MBHA resin that had been preswollen overnight in DCM. The resin was initially substituted with approximately 0.25 mmol/g Boc-Lys(ClZ) as determined by quantitative ninhydrin reaction. The stepwise synthesis of the oligomer followed synthetic Protocol 9 employing 0.023 g (0.06 mmol) BocTaeg-OH, 0.062 g (0.12 mmol) BocbC(Z)-OH and 0.012 g (0.06 mmol) DCC in 1.2 ml 50% DMF/CH_2Cl_2 in each coupling. Capping of uncoupled amino groups was carried out before deprotection in each step. All qualitative Kaiser tests were negative, indicating near 100% coupling yield. The PNA-oligomer was cleaved and purified by standard procedures.

EXAMPLE 81

Hybridizati n properti s of H-T_4bCT_5-Lys-NH_2

Oligodeoxynucleotide	Tm(°C)
5'-AAA AAA AAA A	43.5
5-'AAA AGA AAA A	58.0
5'-AAA AAG AAA A	60.0
5'-AAA ACA AAA A	34.5
5'-AAA AAC AAA A	34.5
5'-AAA ATA AAA A	34.0
5'-AAA AAT AAA A	36.0

EXAMPLE 82

Stepwise Assembly of H-[Taeg]-[Taeg]-[Taeg]-[Taeg]-[Aaeg]-[Taeg]-[Taeg]-[Taeg]-[Taeg]-[Taeg]-LYS-NH_2.

Synthesis was initiated on a Boc-$[Taeg]_5$-Lys(ClZ)-MBHA resin (from example 76) that had been preswollen overnight in DCM. The resin resembled approximately 100 mg (dry Weight) of Boc-Lys(ClZ)-MBHA resin (loading 0.15 mmol/g). The incorporation of the monomers followed the protocol of example 55, except for step 5 (incorporation of the BocA(Z)aeg-OH monomer). New step 5 (incorporation of A(Z)aeg) involved addition of 4 equiv. diisopropyl carbodiimide (0.06 mmol; 9.7 µl) and 4 equiv. BocA(Z)aeg-OH (0.06 mmol; 32 mg) dissolved in 0.6 ml DCM/DMF (1:1, v/v) (final concentration of monomer 0.1 M). The coupling reaction was allowed to proceed for 1 x 15 min. and 1 x 60 min. (recoupling).

Capping of uncoupled amino groups was only carried out before the incorporation of the BocA(Z)aeg-OH monomer. The coupling reaction was monitored by qualitative ninhydrin reaction (Kaiser test). All qualitative Kaiser tests were negative (straw-yellow color with no coloration of the beads). The PNA oligomer was cleaved and purified by standard procedures.

EXAMPLE 84

Hybridizati n properties f H-T$_4$AT$_5$-LysNH$_2$

Oligodeoxynucleotide	Tm(°C)
5′-AAA AAA AAA A	59.5
5-′AAA AGA AAA A	45.0
5′-AAA AAG AAA A	45.5
5′-AAA ACA AAA A	48.0
5′-AAA AAC AAA A	48.0
5′-AAA ATA AAA A	52.0
5′-AAA AAT AAA A	52.5

EXAMPLE 85

Stepwise Assembly of H-[Taeg]-[Taeg]-[Taeg]-[Taeg]-[Gaeg]-[Gaeg]-[Taeg]-[Gaeg]-[Taeg]-[Gaeg]-LYS-NH$_2$.

The protected PNA was assembled onto a Boc-Lys(ClZ) modified MBHA resin with a substitution of 0.15 mmol/g. The incorporation of the monomers followed the protocol of example 32, except that the capping step 11 and the washing step 12 were omitted. After the incorporation and deprotection of the first, second, and fourth G(Bzl)aeg-monomer there were some difficulties getting the resin to swell properly. Three hours of shaking in neat DCM gave acceptable swelling. For the incorporation of residues Taeg-4, G(Bzl)aeg-6, and Taeg-7 to Taeg-10, recoupling was necessary to obtain near quantitativ coupling yields. Taeg$_4$ (2 x in 50% DMF/DCM),Gaeg$_6$ (2 x in 50% DMF/DCM), Taeg$_7$ (2 x in 50% DMF/DCM, 1 x in 50% NMP/DCM and 1 x in neat DCM), Taeg$_8$ (1 x in 50% DMF/DCM and 2 x in neat DCM), Taeg$_9$ (2 x in 50% DMF/DCM), Taeg$_{10}$ (2 x in 50% DMF/DCM). All qualitative Kaiser tests were negative (straw-yellow color with no coloration of the beads). The PNA oligomer was cleaved and purified by standard procedures

EXAMPLE 86

Hybridizati n pr perti s f crude (appr x. 50%) H-T_4G_2TGTG-$LysNH_2$

Oligodeoxynucleotide	Tm
5'-A4C2ACAC	38
5'-CACAC2A4	55

EXAMPLE 87

Large Scale Solid-Phase Synthesis of H-$[Taeg]_6$-Lys-NH_2, H-$[Taeg]_7$-Lys-NH_2, H-$[Taeg]_8$-Lys-NH_2, H-$[Taeg]_9$-Lys-NH_2, and H-$[Taeg]_{10}$-Lys-NH_2.

(a) Stepwise Assembly of Boc-$[Taeg]_{10}$-Lys(ClZ)-MBHA Resin and Shorter Fragments.

About 9 g of wet Boc-$[Taeg]_3$-Lys(ClZ)-MBHA (*see*, Example 19b) resin was placed in a 60 ml SPPS reaction vessel. Boc-$[Taeg]_5$-Lys(ClZ)-MBHA resin was assembled by single coupling of both residues with 0.15 M of BocTaeg-OPfp in 10 ml neat CH_2Cl_2 ("Synthetic Protocol 8"). Both coupling reactions were allowed to proceed overnight. The synthesis was monitored by the ninhydrin reaction, which showed close to quantitative incorporation of both residues. After deprotection of the N-terminal Boc group, about 4.5 g of H-$[Taeg]_5$-Lys(ClZ)-MBHA was placed in a 20 ml SPPS reaction vessel and elongated to Boc-$[Taeg]_8$-Lys(ClZ)-MBHA by single *in situ* DCC coupling of all residues (close to quantitative, except for residue number eight) overnight with 0.2 M of BocTaeg-OH together with 0.2 M DCC in 7.5 ml neat CH_2Cl_2 ("Synthetic Protocol 9"). Before coupling of Taeg residues number seven and eight, respectively, small portions of H-$[Taeg]_6$-Lys(ClZ)-MBHA and H-$[Taeg]_7$-Lys(ClZ)-MBHA, respectively, were taken out for HF cleavage.

Taeg residue number eight was coupled twice (v rnight) to give close to quantitative incorp ration. After deprotection of the N-terminal Boc gr up, a large portion of H-$[Taeg]_8$-Lys(ClZ)-MBHA was taken out for HF cleavag . B c-

[Taeg]$_{10}$-Lys(ClZ)-MBHA resin was assembled by d uble *in situ* DCC coupling of 0.16 M BocTaeg-OH, together with 0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 ("Synthetic Protocol" 9). Before coupling of the final residue, a small portion of H-[Taeg]$_9$-Lys(ClZ)-MBHA was taken out for HF cleavage.

(b) Cleavage, Purification, and Identification of H-[Taeg]$_6$-Lys-NH_2.

The protected Boc-[Taeg]$_6$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 14.0 mg of crude material upon HF cleavage of 52.4 mg dry H-Taeg]$_6$-Lys(ClZ)-MBHA resin. The crude product was not purified (about 99% purity).

(c) Cleavage, Purification, and Identification of H-[Taeg]$_7$-Lys-NH_2.

The protected Boc-[Taeg]$_7$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 5.2 mg of crude material upon HF cleavage of 58.4 mg dry H-Taeg]$_7$-Lys(ClZ)-MBHA resin.

(d) Cleavage, Purification, and Identification of H-[Taeg]$_8$-Lys-NH_2.

The protected Boc-[Taeg]$_8$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 114 mg of crude material upon HF cleavage of about 604 mg dry H-Taeg]$_8$-Lys(ClZ)-MBHA resin.

(e) Cleavage, Purification, and Identification of H-[Taeg]$_9$-Lys-NH_2.

The protected Boc-[Taeg]$_9$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 19.3 mg of crude material upon HF cleavage of 81.0 mg dry H-Taeg]$_9$-Lys(ClZ)-MBHA resin.

(f) Cleavage, Purification, and Identification of H-[Taeg]$_{10}$-Lys-NH_2.

The protected Boc-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin was treated as d scribed in Example 17c to yield about 141 mg of crude material upon HF cleavage f ab ut 417 mg dry H-Taeg]$_{10}$-Lys(ClZ)-MBHA r sin.

(g) Synth tic Protoc l 8 (General Protocol)

(1) Boc-d pr tection with TFA/CH_2Cl_2 (1:1, v/v), 3 x 1 min and 1 x 30 min; (2) washing with CH_2Cl_2, 6 x 1 min; (3) neutralization with $DIEA/CH_2Cl_2$ (1: 19, v/v), 3 x 2 min; (4) washing with CH_2Cl_2, 6 x 1 min, and drain for 1 min; (5) at some stages of the synthesis, 2-5 mg sample of PNA-resin is taken out and dried thoroughly for a ninhydrin analysis to determine the substitution; (6) addition of Boc-protected PNA monomer (Pfp ester); the coupling reaction was allowed to proceed for a total of X hrs shaking; (7) washing with DMF, 1 x 2 min; (8) washing with CH_2Cl_2, 4 x 1 min; (9) neutralization with $DIEA/CH_2Cl_2$ (1: 19, v/v), 2 x 2 min; (10) washing with CH_2Cl_2, 6 x 1 min; (11) occasionally, 2-5 mg sample of protected PNA-resin is taken out and dried thoroughly for a ninhydrin analysis to determine the extent of coupling; (12) at some stages of the synthesis, unreacted amino groups are blocked by acetylation with a mixture of acetic anhydride/pyridine/CH_2Cl_2 (1:1:2, v/v/v) for 2 h followed by washing with CH_2Cl_2, 6 x 1 min, and, occasionally, ninhydrin analysis.

EXAMPLE 88

Solid-Phase Synthesis of H-[Taeg]4-Caeg-[Taeg]5-Lys-NH_2.

(a) Stepwise Assembly of Boc-[Taeg]4-C[Z]aeg-[Taeg]5-Lys(ClZ)-MBHA Resin.

About 1 g of wet Boc-[Taeg]5-Lys(ClZ)-MBHA resin was placed in a 5 ml SPPS reaction vessel. Boc-[Taeg]4-C[Z]aeg-[Taeg]5-Lys(ClZ)-MBHA resin was assembled by *in situ* DCC coupling of all residues utilizing 0.16 M of BocC[Z]aeg-OH together with 0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 or 0.16 M BocTaeg-OH together with 0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 ("Synthetic Protocol 9"). Each coupling reaction was allowed to proceed for a total of 20-24 hrs with shaking. The synthesis was monitored by the ninhydrin reaction, which showed ab ut 98% incorporation of C[Z]aeg and close to quantitative inc rp rati n of all the Taeg residues.

(b) Cl avag , Purification, and Id ntificati n f H-[Taeg]4-C[Z]aeg-[Taeg]5-Lys-NH_2.

The protected Boc-[Taeg]4-C[Z]aeg-[Taeg]5-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 22.5 mg of crude material upon HF cleavage of 128.2 mg dry H-[Taeg]4-C[Z]aeg-[Taeg]5-Lys(ClZ)-MBHA resin. Crude product (5.8 mg) was purified to give 3.1 mg of H-[Taeg]4-Caeg-[Taeg]5-Lys-NH_2.

(c) Synthetic Protocol 9 (General Protocol)

(1) Boc-deprotection with TFA/CH_2Cl_2 (1:1, v/v), 3 x 1 min and 1 x 30 min; (2) washing with CH_2Cl_2, 6 x 1 min; (3) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 3 x 2 min; (4) washing with CH_2Cl_2, 6 x 1 min, and drain for 1 min; (5) at some stages of the synthesis, 2-5 mg sample of PNA-resin is taken out and dried thoroughly for a ninhydrin analysis to determine the substitution; (6) addition of Boc-protected PNA monomer (free acid) in X ml DMF followed by addition of DCC in X ml CH_2Cl_2; the coupling reaction was allowed to proceed for a total of Y hrs shaking; (7) washing with DMF, 1 x 2 min; (8) washing with CH_2Cl_2, 4 x 1 min; (9) neutralization with DIEA/CH_2Cl_2 (1: 19, v/v), 2 x 2 min; (10) washing with CH_2Cl_2, 6 x 1 min; (11) occasionally, 2-5 mg sample of protected PNA-resin is taken out and dried thoroughly for a ninhydrin analysis to determine the extent of coupling; (12) at some stages of the synthesis, unreacted amino groups are blocked by acetylation with a mixture of acetic anhydride/pyridine/CH_2Cl_2 (1:1:2, v/v/v) for 2 h followed by washing with CH_2Cl_2, 6 x 1 min, and, occasionally, ninhydrin analysis.

EXAMPLE 89

Solid-Phase Synthesis of H-[Taeg]4-(NBaeg)-[Taeg]5-Lys-NH_2. (NB = COCH3)

(a) Stepwise Assembly of Boc-[Taeg]4-(NBaeg)-[Ta g]5-Lys(ClZ)-MBHA Resin.

About 1 g of wet Boc-[Taeg]5-Lys(ClZ)-MBHA resin was placed in a 5 ml SPPS reaction vessel. Boc-[Taeg]4-(NBaeg)-

[Taeg]5-Lys(ClZ)-MBHA resin was assembled by *in situ* DCC coupling utilizing 0.16 M of Boc(NBaeg)-OH t gether with 0.16 M DCC in 2.0 ml neat CH_2Cl_2 or 0.16 M BocTaeg-OH together with 0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 ("Synthetic Protocol 9"). Each coupling reaction was allowed to proceed for a total of 20-24 hrs with shaking. The NBaeg residue was coupled three times and the Taeg residues were all coupled once. The synthesis was monitored by the ninhydrin reaction which showed >99% total incorporation of NBaeg (about 88% after the first coupling and about 93% after the second coupling) and close to quantitative incorporation of all the Taeg residues.

(b) Cleavage, Purification, and Identification of H-[Taeg]4-(NBaeg)-[Taeg]5-Lys-NH_2.

The protected Boc-[Taeg]4-(NBaeg)-[Taeg]5-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 33.6 mg of crude material upon HF cleavage of 108.9 mg dry H-[Taeg]4-(NBaeg)-[Taeg]5-Lys(ClZ)-MBHA resin. Crude product (20.6 mg) was purified to give 4.6 mg of H-[Taeg]4-(NBaeg)-[Taeg]5-Lys-NH_2. For (M+H)+, the calculated m/z value was 2683.12 and the measured m/z value was 2683.09.

EXAMPLE 90

Solid-Phase Synthesis of H-[Taeg]4-aeg-[Taeg]5-Lys-NH_2.

(a) Stepwise Assembly of Boc-[Taeg]4-aeg-[Taeg]5-Lys(ClZ)-MBHA Resin.

About 1 g of wet Boc-[Taeg]5-Lys(ClZ)-MBHA resin was placed in a 5 ml SPPS reaction vessel. Boc-[Taeg]4-aeg-[Taeg]5-Lys(ClZ)-MBHA resin was assembled by *in situ* DCC single coupling of all residues utilizing: (1) 0.16 M of Bocaeg-OH together with 0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 or (2) 0.16 M BocTaeg-OH together with (2) 0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 ("Synthetic Protocol 9"). Each coupling reaction was allowed to proceed for a total of 20-24 hrs with shaking. The synthesis was monitored by the ninhydrin reaction, which showed close to quantitative incorporation of all the residues.

(b) Cl avage, Purificati n, and Identification f H-[Taeg]4-aeg-[Taeg]5-Lys-NH_2.

The protected Boc-[Taeg]4-aeg-[Taeg]5-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 22.2 mg of crude material upon HF cleavage of 126.0 mg dry H-[Taeg]4-aeg-[Taeg]5-Lys(ClZ)-MBHA resin. Crude product (22.2 mg) was purified to give 7.6 mg of H-[Taeg]4-aeg-[Taeg]5-Lys-NH_2. For (M+H)+, the calculated m/z value was 2641.11 and the measured m/z value was 2641.16.

EXAMPLE 91

Solid-Phase Synthesis of H-[Taeg]4-Gly-[Taeg]5-Lys-NH_2.

(a) Stepwise Assembly of Boc-[Taeg]4-Gly-[Taeg]5-Lys(ClZ)-MBHA Resin.

About 1 g of wet Boc-[Taeg]5-Lys(ClZ)-MBHA resin was placed in a 5 ml SPPS reaction vessel. Boc-[Taeg]4-Gly-[Taeg]5-Lys(ClZ)-MBHA resin was assembled by *in situ* DCC single coupling of all residues utilizing: (1) 0.16 M of BocGly-OH together with 0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 or (2) 0.16 M BocTaeg-OH together with 0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 ("Synthetic Protocol 9"). Each coupling reaction was allowed to proceed for a total of 20-24 hrs with shaking. The synthesis was monitored by the ninhydrin reaction, which showed close to quantitative incorporation of all the residues.

(b) Cleavage, Purification, and Identification of H-[Taeg]4-Gly-[Taeg]5-Lys-NH_2.

The protected Boc-[Taeg]4-Gly-[Taeg]5-Lys(ClZ)-MBHA resin was treated as described in Example 18c to yield about 45.0 mg of crude material upon HF cleavage of 124.1 mg dry H-[Taeg]4-Gly-[Taeg]5-Lys(ClZ)-MBHA resin. Crude product (40.4 mg) was purified to give 8.2 mg of H-[Taeg]4-Gly-[Taeg]5-Lys-NH_2.

EXAMPLE 92

S lid-Phas Synth sis f H-[Taeg]4-Gly2-[Taeg]5-Lys-NH_2.

(a) Stepwise Assembly of Boc-[Taeg]4-Gly2-[Taeg]5-Lys(ClZ)-MBHA Resin.

About 1 g of wet Boc-[Taeg]5-Lys(ClZ)-MBHA resin was placed in a 5 ml SPPS reaction vessel. Boc-[Taeg]4-[C[Z]aeg]2-Taeg-C[Z]aeg-Taeg-C[Z]aeg-Lys(ClZ)-MBHA resin was assembled by *in situ* DCC single coupling of all residues utilizing: (1) 0.16 M of BocGly-OH together with 0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 or (2) 0.16 M BocTaeg-OH together with 0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 ("Synthetic Protocol 9"). Each coupling reaction was allowed to proceed for a total of 20-24 hrs with shaking. The synthesis was monitored by the ninhydrin reaction, which showed close to quantitative incorporation of all the residues.

(b) Cleavage, Purification, and Identification of H-[Taeg]4-Gly2-[Taeg]5-Lys-NH_2.

The protected Boc-[Taeg]4-Gly2-[Taeg]5-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 32.6 mg of crude material upon HF cleavage of 156.6 mg dry H-[Taeg]4-Gly2-[Taeg]5-Lys(ClZ)-MBHA resin. Crude product (30 mg) was purified to give 7.8 mg of H-[Taeg]4-Gly2-[Taeg]5-Lys-NH_2. For (M+H)+, the calculated m/z value was 2655.09 and the measured m/z value was 2655.37.

EXAMPLE 93

S lid-Phas Synthesis of H-[Taeg]4-[Caeg]2-Taeg-Ca g-Ta g-Caeg-Lys-NH_2.

(a) Stepwise Assembly of Boc-[Taeg]4-[C[Z]aeg]2-Taeg-C[Z]aeg-Taeg-C[Z]aeg-Lys(ClZ)-MBHA Resin.

About 1.5 g of wet Boc-Lys(ClZ)-MBHA (0.28 mmol.Lys/g) resin was placed in a 5 ml SPPS reaction vessel. Boc-[Taeg]4-[C[Z]aeg]2-Taeg-C[Z]aeg-Taeg-C[Z]aeg-Lys(ClZ)-MBHA resin was assembled by *in situ* DCC single coupling of all residues utilizing: (1) 0.16 M of BocC[Z]-OH together with 0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 or (2) 0.16 M BocTaeg-OH together with 0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 ("Synthetic Protocol 9"). Each coupling reaction was allowed to proceed for a total of 20-24 hrs with shaking. The synthesis was monitored by the ninhydrin reaction, which showed close to quantitative incorporation of all the residues.

(b) Cleavage, Purification, and Identification of H-$[Taeg]_4$-$[Caeg]_2$-Taeg-Caeg-Taeg-Caeg-Lys-NH_2.

The protected Boc-[Taeg]4-[C[Z]aeg]2-Taeg-C[Z]aeg-Taeg-C[Z]aeg-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 52.1 mg of crude material upon HF cleavage of 216.7 mg dry H-[Taeg]4-[C[Z]aeg]2-Taeg-C[Z]aeg-Taeg-C[Z]aeg-Lys(ClZ)-MBHA resin. Crude product (30.6 mg) was purified to give 6.2 mg of H-[Taeg]4-[Caeg]2-Taeg-Caeg-Taeg-Caeg-Lys-NH_2. For (M+H)+ the calculated m/z value was 2747.15 and the measured m/z value was 2746.78.

EXAMPLE 94

Solid-Phase Synthesis of H-Caeg-Taeg-Caeg-Taeg-[Caeg]3-Taeg-Caeg-Taeg-Lys-NH_2.

(a) Stepwise Assembly of Boc-C[Z]aeg-Taeg-C[Z]aeg-Taeg-[C[Z]aeg]3-Taeg-C[Z]aeg-Taeg-Lys(ClZ)-MBHA Resin.

About 1.5 g of wet Boc-Lys(ClZ)-MBHA (0.28 mmol Lys/g) resin was placed in a 5 ml SPPS reaction vessel. B c-C[Z]aeg-Taeg-C[Z]aeg-Taeg-[C[Z]aeg]3-Taeg-C[Z]aeg-Taeg-Lys(ClZ)-MBHA resin was assembled by *in situ* DCC single coupling of all residues utilizing: (1) 0.16 M of B cC[Z]-OH together with

0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 r (2) 0.16 M BocTaeg-OH together with 0.16 M DCC in 2.0 ml 50% DMF/CH_2Cl_2 ("Synthetic Protocol 9"). Each coupling reaction was allowed to proceed for a total of 20-24 hrs with shaking. The synthesis was monitored by the ninhydrin reaction, which showed close to quantitative incorporation of all the residues.

(b) Cleavage, Purification, and Identification of H-Caeg-Taeg-Caeg-Taeg-[Caeg]3-Taeg-Caeg-Taeg-Lys-NH 2.

The protected Boc-C[Z]aeg-Taeg-C[Z]aeg-Taeg-[C[Z]aeg]3-Taeg-C[Z]aeg-TaegLys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 56.1 mg of crude material upon HF cleavage of 255.0 mg dry H-C[Z]aeg-Taeg-C[Z]aeg-Taeg-[C[Z]aeg]3-Taeg-C[Z]aeg -TaegLys(ClZ)-MBHA resin. Crude product (85.8 mg) was purified to give 46.2 mg of H-Caeg-Taeg-Caeg-Taeg-[Caeg]3-Taeg-Caeg-Taeg-LysNH$_2$. For (M+H)+ the calculated m/z value was 2717.15 and the measured m/z value was 2716.93.

EXAMPLE 95

Solid-Phase Synthesis of H-[Taeg]2-[Caeg]3-[Taeg]2-[Caeg]2-Lys-NH$_2$, H-Caeg-[Taeg]2-[Caeg]3-[Taeg]2-[Caeg]2-Lys-NH$_2$, and H-Tyr-[Taeg]2-[Caeg]3-[Taeg]2-[Caeg]2-Lys-NH$_2$.

(a) Stepwise Assembly of Boc-[Taeg]2-[C(Z)aeg]3-[Taeg]2-[C(Z)aeg]2-Lys(ClZ)-MBHA Resin, Boc-Caeg-[Taeg]2-[C(Z)aeg]3-[Taeg]2-[C(Z)aeg]2-Lys(ClZ)-MBHA Resin, and Boc-Tyr(BrZ)-[Taeg]2-[C(Z)aeg]3-[Taeg]2-[C(Z)aeg]2-Lys(ClZ)-MBHA Resin.

About 3 g of wet Boc-Lys(ClZ)-MBHA (0.28 mmol Lys/g) resin was placed in a 20 ml SPPS reaction vessel. Boc-[Taeg]2-[C(Z)aeg]3-[Taeg]2-[C(Z)aeg]2-Lys(ClZ)-MBHA resin was assembled by *in situ* DCC single coupling of all residues utilizing: (1) 0.16 M of BocC[Z]-OH together with 0.16 M DCC in 3.0 ml 50% DMF/CH_2Cl_2 or (2) 0.16 M BocTaeg-OH together with 0.16 M DCC in 3.0 ml 50% DMF/CH_2Cl_2 ("Synthetic Protocol 9"). Each coupling reaction was allowed to proceed f r a total of 20-24 hrs with shaking. The synthesis was monitored by the ninhydrin reacti n, which showed close to quantitativ

inc rporati n of all the residues. After deprotecti n of the N-terminal Boc group, half of the PNA-resin was coupled quantitatively onto Tyr(BrZ)-OH and a small portion was coupled quantitatively onto one more Caeg residue. Both couplings employed the above-mentioned synthetic protocol.

(b) Cleavage, Purification, and Identification of H-[Taeg]2-[Caeg]3-[Taeg]2-[Caeg]2-Lys-NH_2.

The protected Boc-[Taeg]2-[C(Z)aeg]3-[Taeg]2-[C(Z)aeg]2-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 50.9 mg of crude material upon HF cleavage of 182.5 mg dry H-[Taeg]2-[C(Z)aeg]3-[Taeg]2-[C(Z)aeg]2-Lys(ClZ)-MBHA resin. Crude product (50.9) mg was purified to give 13.7 mg of H-[Taeg]2-[Caeg]3-[Taeg]2-[Caeg]2-LysNH_2. For (M+H)+ the calculated m/z value was 2466.04; the m/z value was not measured.

(c) Cleavage, Purification, and Identification of H-Tyr-[Taeg]2-[Caeg]3-[Taeg]2-[Caeg]2-Lys-NH_2.

The protected Boc-Tyr(BrZ)-[Taeg]2-[C(Z)aeg]3-[Taeg]2-[C(Z)aeg]2-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 60.8 mg of crude material upon HF cleavage of 188.8 mg dry H-Tyr(BrZ)-[Taeg]2-[C(Z)aeg]3-[Taeg]2-[C(Z)aeg]2-Lys(ClZ)-MBHA resin. Crude product (60.8 mg) was purified to give 20.7 mg of H-Tyr-[Taeg]2-[Caeg]3-[Taeg]2-[Caeg]2-LysNH_2. For (M+H)+ the calculated m/z value was 2629.11 and the measured m/z value was 2629.11.

(d) Cleavage, Purification, and Identification of H-Caeg-[Taeg]2-[Caeg]3-[Taeg]2-[Caeg]2-Lys-NH_2.

The protected Boc-C(Z)aeg-[Taeg]2-[C(Z)aeg]3-[Taeg]2-[C(Z)aeg]2-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 11.7 mg of crude material upon HF cleavage of 42.0 mg dry H-C(Z)aeg-[Taeg]2-[C(Z)aeg]3-[Taeg]2-[C(Z)aeg]2-Lys(ClZ)-MBHA resin. Crude product (11.6 mg) was purified to give 3.1 mg of H-Caeg-[Taeg]2-[Caeg]3-[Taeg]2-[Caeg]2-LysNH_2. For (M+H)+ the calculated m/z value was 2717.15; the m/z value was n t measur d.

EXAMPLE 96

S lid-Phas Synth sis f H-[Caeg]2-[Ta g]2-[Caeg]3-[Taeg]2-Lys-NH_2,
H-Taeg-[Caeg]2-[Taeg]2-[Caeg]3-[Taeg]2-Lys-NH_2, and H-Tyr-[Caeg]2-[Taeg]2-[Caeg]3-[Taeg]2-Lys-NH_2.

(a) Stepwise Assembly of Boc-[C(Z)aeg]2-[Taeg]2-[C(Z)aeg]3-[Taeg]2-Lys(ClZ)-MBHA Resin, Boc-Taeg-[C(Z)aeg]2-[Taeg]2-[C(Z)aeg]3-[Taeg]2-Lys(ClZ)-MBHA Resin, and Boc-Tyr(BrZ)-[C(Z)aeg]2-[Taeg]2-[C(Z)aeg]3-[Taeg]2-Lys(ClZ)-MBHA Resin.

About 3 g of wet Boc-Lys(ClZ)-MBHA (0.28 mmol Lys/g) resin was placed in a 20 ml SPPS reaction vessel. Boc-[Taeg]2-[C(Z)aeg]3-[Taeg]2-[C(Z)aeg]2-Lys(ClZ)- MBHA resin was assembled by *in situ* DCC single coupling of all residues utilizing: (1) 0.16 M of BocC[Z]-OH together with 0.16 M DCC in 3.0 ml 50% DMF/CH_2Cl_2 or (2) 0.16 M BocTaeg-OH together with 0.16 M DCC in 3.0 ml 50% DMF/CH_2Cl_2 ("Synthetic Protocol 9"). Each coupling reaction was allowed to proceed for a total of 20-24 hrs with shaking. The synthesis was monitored by the ninhydrin reaction, which showed close to quantitative incorporation of all the residues. After deprotection of the N-terminal Boc group, half of the PNA-resin was coupled quantitatively onto Tyr(BrZ)-OH and a small portion was coupled quantitatively onto one more Taeg residue. Both couplings employed the above-mentioned synthetic protocol.

(b) Cleavage, Purification, and Identification of H-[C(Z)aeg]2-[Taeg]2-[C(Z)aeg]3-[Taeg]2-Lys-NH_2.

The protected Boc-[C(Z)aeg]2-[Taeg]2-[C(Z)aeg]3-[Taeg]2-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 57.6 mg of crude material upon HF cleavage of 172.7 mg dry H-[C(Z)aeg]2-[Taeg]2-[C(Z)aeg]3-[Taeg]2-Lys(ClZ)-MBHA resin. Crude product (57.6 mg) was purified to give 26.3 mg of H-[Caeg]2-[Taeg]2-[Caeg]3-[Taeg]2-Lys-NH_2. For (M+H)+ the calculated m/z value was 2466.04; the m/z value was n t measured.

(c) Cleavag , Purificati n, and Id ntificati n f H-Tyr-[C(Z)aeg]2-[Taeg]2-[C(Z)aeg]3-[Taeg]2-Lys-NH_2 .

The protected Boc-Tyr(BrZ)-[C(Z)aeg]2-[Taeg]2-[C(Z)aeg]3-[Taeg]2-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 57.6 mg of crude material upon HF cleavage of 172.7 mg dry H-Tyr(BrZ)-[C(Z)aeg]2-[Taeg]2-[C(Z)aeg]3-[Taeg]2-Lys(ClZ)-MBHA resin. Crude product (47.1 mg) was purified to give 13.4 mg of H-Tyr-[Caeg]2-[Taeg]2-[Caeg]3-[Taeg]2-Lys-NH_2. For (M+H)+ the calculated m/z value was 2629.11 and the measured m/z value was 2629.11.

(d) Cleavage, Purification, and Identification of H-Taeg-[C(Z)aeg]2-[Taeg]2-[C(Z)aeg]3-[Taeg]2-Lys-NH_2.

The protected Boc-Taeg-[C(Z)aeg]2-[Taeg]2-[C(Z)aeg]3-[Taeg]2-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 53.4 mg of crude material upon HF cleavage of 42.4 mg dry H-Taeg-[C(Z)aeg]2-[Taeg]2-[C(Z)aeg]3-[Taeg]2-Lys(Cl Z)-MBHA resin. Crude product (11.9 mg) was purified to give 4.3 mg of H-Taeg-[Caeg]2-[Taeg]2-[Caeg]3-[Taeg]2-Lys-NH_2. For (M+H)+ the calculated m/z value was 2732.15; the m/z value was not measured.

(c) Synthetic Protocol 10 (General Protocol)

Same protocol as "Synthetic Protocol 9", except that DCC has been replaced with DIC.

EXAMPLE 97

SYNTHESIS OF THE BACKBONE MOIETY FOR SCALE UP BY REDUCTIVE AMINATION

(a) Preparation of (bocamino)acetaldehyde.

3-Amino-1,2-propanediol(80.0 g; 0.88 mol) was dissolved in water (1500 ml) and the solution was cooled to 4°C, whereafter Boc anhydride (230 g; 1.05 mol) was added at once. The solution was gently heated to room temperature with a water bath. The pH was kept at 10.5 by the dropwise addition of sodium hydr xide. Over the course of the reaction a total f 70.2 g NaOH, dissolved in 480 ml water, was added. After stirring ov rnight, thyl acetate (1000 ml) was added and th

mixtur was c oled t 0°C and the pH was adjusted t 2.5 by the addition of 4 M hydr chloric acid. The ethyl acetat layer was removed and the acidic aqueous solution was extracted with more ethyl acetate (8x500 ml). The combined ethyl acetate solution was reduced to a volume of 1500 ml using a rotary evaporator. The resulting solution was washed with half saturated potassium hydrogen sulphate (1500 ml) and then with saturated sodium chloride. It then was dried over magnesium sulphate and evaporated to dryness, *in vacuo*. Yield. 145.3 g (86%)

3-Bocamino-1,2-propanediol (144.7 g; 0.757 mol) was suspended in water (750 ml) and potassium periodate (191.5 g; 0.833 mol) was added. The mixture was stirred under nitrogen for 2.5 h and the precipitated potassium iodate was removed by filtration and washed once with water (100 ml). The aqueous phase was extracted with chloroform (6x400 ml). The chloroform extracts were dried and evaporated to dryness, *in vacuo*. Yield 102 g (93%) of an oil. The (bocamino)acetaldehyde was purified by kugelrohr distillation at 84°C and 0.3 mmHg in two portions. The yield 79 g (77%) of a colorless oil.

(b) Preparation of (N′-bocaminoethyl)glycine methyl ester

Palladium on carbon (10%; 2.00 g) was added to a solution of (bocamino)acetaldehyde (10.0 g; 68.9 mmol) in methanol (150 ml) at 0°C. Sodium acetate (11.3 g; 138 mmol) in methanol (150 ml), and glycine methyl ester hydrochloride (8.65 g; 68.9 mmol) in methanol (75 ml) then were added. The mixture was hydrogenated at atmospheric pressure for 2.5 h, then filtered through celite and evaporated to dryness, *in vacuo*. The material was redissolved in water (150 ml) and the pH was adjusted to 8.0 with 0.5 N NaOH. The aqueous solution was extracted with methylene chloride (5 x 150 ml). The combined extracts were dried over sodium sulphate and evaporated to dryness, *in vacuo*. This resulted in 14.1 g (88%) of (N′-bocaminoethyl)glycine methyl ester. The crud material was purified by kug lrohr destination at 120°C and

0.5 mmHg to give 11.3 g (70%) of a colorless il. The product had a purity that was higher than the material pr duced in example 26 according to tlc-analysis (10% methanol in methylene chloride).

Alternatively, sodium cyanoborohydride can be used as reducing agent instead of hydrogen (with Pd(C) as catalyst), although the yield (42%) was lower.

(c) Preparation of (N′-bocaminoethyl)glycine ethyl ester.

The title compound was prepared by the above procedure with glycine ethyl ester hydrochloride substituted for glycine methyl ester hydrochloride. Also, the solvent used was ethanol. The yield was 78%.

EXAMPLE 98

Solid-Phase Synthesis of H-Tyr-[Taeg]$_{10}$-Lys-NH_2

(a) Stepwise Assembly of Boc-Tyr(BrZ)-[Taeg]$_{10}$-Lys(ClZ)-MBHA Resin.

About 0.2 g of wet Boc-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin was placed in a 5 ml SPPS reaction vessel. Boc-Tyr(BrZ)-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin was assembled by standard *in situ* DCC coupling utilizing 0.32 M of BocCTyr(BrZ)-OH together with 0.32 M DCC in 3.0 ml neat CH_2Cl_2 overnight. The ninhydrin reaction showed about 97% incorporation of BocTyr(BrZ).

(b) Cleavage, Purification, and Identification of H-Tyr-[Taeg]$_{10}$-Lys-NH_2.

The protected Boc-Tyr(BrZ)-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 5.5 mg of crude material upon HF cleavage of 20.7 mg dry H-Tyr(BrZ)-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin. The crude product was purified to give 2.5 mg of H-Tyr-[Taeg]$_{10}$-Lys-NH_2.

EXAMPLE 99

S lid-Phase Synthesis f Dansyl-[Taeg]$_{10}$-Lys-NH_2

(a) Stepwise Assembly of Dansyl-[Taeg]$_{10}$-Lys(ClZ)-MBHA Resin.

About 0.3 g of wet Boc-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin was placed in a 5 ml SPPS reaction vessel. Dansyl-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin was assembled by coupling of 0.5 M dansyl-Cl in 2.0 ml neat pyridine overnight. The ninhydrin reaction showed about 95% incorporation of dansyl.

(b) Cleavage, Purification, and Identification of Dansyl-[Taeg]$_{10}$-Lys-NH_2.

The protected dansyl-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 12 mg of crude material upon HF cleavage of 71.3 mg dry dansyl-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin. The crude product was purified to give 5.4 mg of dansyl-[Taeg]$_{10}$-Lys-NH_2.

EXAMPLE 100

Solid-Phase Synthesis of Gly-Gly-His-[Taeg]$_{10}$-Lys-NH_2

(a) Stepwise Assembly of Boc-Gly-Gly-His(Tos)-[Taeg]$_{10}$-Lys(ClZ)-MBHA Resin.

About 0.05 g of Boc-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin was placed in a 5 ml SPPS reaction vessel. Boc-Gly-Gly-His(Tos)-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin was assembled by standard double *in situ* DCC coupling of Boc-protected amino acid (0.1 M) in 2.5 ml 25% DMF/CH_2Cl_2, except for the first coupling of BocHis(Tos), which was done by using a preformed symmetrical anhydride (0.1M) in 25% DMF/CH_2Cl_2. All couplings were performed overnight and ninhydrin reactions were not carried out.

(b) Cleavage, Purification, and Identification of Gly-Gly-His-[Taeg]$_{10}$-Lys-NH_2.

The protected Boc-Gly-Gly-His(Tos)-[Taeg]$_{10}$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield ab ut 10.3 mg of crude material (about 40% purity) upon HF cleavage of 34.5 mg dry Boc-Gly-Gly-His(Tos)-[Taeg]$_{10}$-Lys(ClZ)-

MBHA resin. A small porti n of the crude pr duct (taken out before ly philization) was purified to give 0.1 mg of Gly-Gly-His-[Taeg]$_{10}$-Lys-NH$_2$.

EXAMPLE 101

Solid-Phase Synthesis of H-[Taeg]$_5$-[Caeg]$_2$-NH$_2$.

(a) Stepwise Assembly of Boc-[Taeg]$_5$-[C(Z)aeg]$_2$-MBHA Resin.

About 0.2 g of MBHA resin was placed in a 3 ml SPPS reaction vessel and neutralized. The loading was determined to be about 0.64 mmol/g. BocC(Z)aeg-OPfp was coupled onto the resin using a concentration of 0.13 M in 2.5 ml 25% phenol//CH$_2$Cl$_2$. The ninhydrin analysis showed a coupling yield of about 40%. The remaining free amino groups were acetylated as usual. Boc-[Taeg]$_5$-[C(Z)aeg]$_2$-MBHA resin was assembled by single *in situ* DCC coupling of the next residue utilizing 0.11 M of BocC(Z)aeg-OH together with 0.11 M DCC in 2.5 ml 50% DMF/CH$_2$Cl$_2$ and by coupling with 0.13 M BocTaeg-OPfp in neat CH$_2$Cl$_2$ for the remaining residues ("Synthetic Protocol 8"). Each coupling reaction was allowed to proceed with shaking overnight. The synthesis was monitored by the ninhydrin reaction, which showed close to quantitative incorporation of all the residues.

(b) Cleavage, Purification, and Identification of H-[Taeg]$_5$-[Caeg]$_2$-NH$_2$.

The protected Boc-[Taeg]$_5$-[C(Z)aeg]$_2$-MBHA resin was treated as described in Example 17c to yield about 21.7 mg of crude material (>80% purity) upon HF cleavage of 94.8 mg dry H-[Taeg]$_5$-[C(Z)aeg]$_2$-MBHA resin. Crude product (7.4 mg) was purified to give 2.0 mg of H-[Taeg]$_5$-[Caeg]$_2$-NH$_2$ (>99% purity).

EXAMPLE 102

Solid-Phase Synthesis of H-[Taeg]$_3$-Caeg-[Taeg]$_4$-NH$_2$.

(a) Stepwise Assembly of Boc-[Taeg]$_3$-C(Z)aeg-[Taeg]$_4$-MBHA R sin.

About 0.2 g of the above-mentioned MBHA resin was placed in a 5 ml SPPS reaction vessel and neutraliz d. Boc-

[Taeg]$_3$-C(Z)aeg-[Taeg]$_4$-MBHA resin was assembled by single *in situ* DCC c upling of the C(Z)aeg residue utilizing 0.13 M of BocC[Z]aeg-OH together with 0.13 M DCC in 2.5 ml 50% DMF/CH_2Cl_2 and by coupling the Taeg residues with 0.13 M BocTaeg-OPfp in 2.5 ml neat CH_2Cl_2. Each coupling reaction was allowed to proceed with shaking overnight. The synthesis was monitored by the ninhydrin reaction, which showed close to quantitative incorporation of all the residues.

(b) Cleavage, Purification, and Identification of H-[Taeg]$_3$-Caeg-[Taeg]$_4$-NH_2.

The protected Boc-[Taeg]$_3$-C(Z)aeg-[Taeg]$_4$-MBHA resin was treated as described in Example 17c to yield about 44.4 mg of crude material upon HF cleavage of about 123 mg dry H-[Taeg]$_3$-C(Z)aeg-[Taeg]$_4$-MBHA resin. Crude product (11.0 mg) was purified to give 3.6 mg of H-[Taeg]$_3$-Caeg-[Taeg]$_4$-NH_2.

EXAMPLE 103

Solid-Phase Synthesis of H-Taeg-Caeg-[Taeg]$_8$-LysNH$_2$.

(a) Stepwise Assembly of Boc-Taeg-C(Z)aeg-[Taeg]$_8$-Lys(ClZ)-MBHA Resin.

About 0.3 g of wet Boc-[Taeg]$_8$-Lys(ClZ)-MBHA resin was placed in a 3 ml SPPS reaction vessel. Boc-Taeg-C(Z)aeg-[Taeg]$_8$-Lys(ClZ)-MBHA resin was assembled by single *in situ* DCC coupling overnight of the C(Z)aeg residue ("Synthetic Protocol" 9) utilizing 0.2 M of BocC[Z]aeg-OH together with 0.2 M DCC in 2.5 ml 50% DMF/CH_2Cl_2 (incorporation was about 80% as judged by ninhydrin analysis; remaining free amino groups were acetylated) and by overnight coupling the Taeg residue with 0.15 M BocTaeg-OPfp in 2.5 ml neat CH_2Cl_2 (nearly quantitatively).

(b) Cleavage, Purification, and Identification of H-Taeg-Caeg-[Taeg]$_8$-LysNH$_2$.

The protected Boc-Taeg-C(Z)aeg-[Taeg]$_8$-Lys(ClZ)-MBHA resin was treated as d scribed in Example 17c to yield about 22.3 mg of crude material upon HF cleavage of ab ut 76.5 mg dry H-Taeg-C(Z)aeg-[Ta g]$_8$-Lys(ClZ)-MBHA resin. Crude product

(6.7 mg) was purified to give 2.6 mg f H-Ta g-Caeg-$[Taeg]_8$-$LysNH_2$. For $(M+H)^+$ the calculated m/z value was 2792.15 and the measured m/z value was 2792.21.

EXAMPLE 104

Solid-Phase Synthesis of H-Caeg-$[Taeg]_5$-Lys-NH_2 and H-$[Taeg]_2$-Caeg-$[Taeg]_5$-Lys-NH_2.

(a) Stepwise Assembly of Boc-$[Taeg]_2$-C(Z)aeg-$[Taeg]_5$-Lys(ClZ)-MBHA Resin.

About 0.5 g of wet Boc-$[Taeg]_5$-Lys(ClZ)-MBHA resin was placed in a 5 ml SPPS reaction vessel. Boc-$[Taeg]_2$-C(Z)aeg-$[Taeg]_5$-Lys(ClZ)-MBHA resin was assembled by single *in situ* DCC coupling of all residues utilizing: (1) 0.12 M of BocC[Z]aeg-OH together with 0.12 M DCC in 3.0 ml 50% DMF/CH_2Cl_2 or (2) 0.12 M BocTaeg-OH together with 0.12 M DCC in 3.0 ml 50% DMF/CH_2Cl_2 ("Synthetic Protocol 9"). Each coupling reaction was allowed to proceed overnight with shaking. Th synthesis was monitored by the ninhydrin reaction, which showed close to quantitative incorporation of all th residues. During the synthesis, a small portion of H-C(Z)aeg-$[Taeg]_5$-Lys(ClZ)-MBHA resin was taken out for HF cleavage.

(b) Cleavage, Purification, and Identification of H-Caeg-$[Taeg]_5$-Lys-NH_2.

The protected Boc-C[Z]aeg-$[Taeg]_5$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 3.0 mg of crude material upon HF cleavage of 37.5 mg dry H-C[Z]aeg-$[Taeg]_5$-Lys(ClZ)-MBHA resin. About 0.7 mg of the crude product was purified to give about 0.5 mg of H-Caeg-$[Taeg]_5$-Lys-NH_2.

(c) Cleavage, Purification, and Identification of H-$[Taeg]_2$-Caeg-$[Taeg]_5$-Lys-NH_2.

The protected Boc-$[Taeg]_2$-C[Z]aeg-$[Taeg]_5$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 37.7 mg of crude material upon HF cleavage of 118.6 mg dry H-$[Taeg]_2$-C[Z]aeg-$[Taeg]_5$-Lys(ClZ)-MBHA resin.

EXAMPLE 105

Solid-Phase Synthesis of H-[Caeg]$_5$-Lys-NH$_2$, H-[Caeg]$_6$-Lys-NH$_2$, H-[Caeg]$_8$-Lys-NH$_2$, and H-[Caeg]$_{10}$-Lys-NH$_2$

(a) Stepwise Assembly of Boc-[C(Z)aeg]$_{10}$-Lys(ClZ)-MBHA Resin and Shorter Fragments.

About 5 g of wet Boc-Lys(ClZ)-MBHA resin (substitution = 0.3 mmol Lys/g) was placed in a 30 ml SPPS reaction vessel. Boc-[C(Z)aeg]$_{10}$-Lys(ClZ)-MBHA resin was assembled by single *in situ* DCC coupling of the first three residues with 0.1 M of BocC(Z)aeg-OH together with 0.1 M DCC in 10 ml 50% DMF/CH_2Cl_2 ("Synthetic Protocol 9") and by single *in situ* DIC coupling of the remaining seven residues with 0.1 M of BocC(Z)aeg-OH together with 0.1 M DIC in 10 ml 50% DMF/CH_2Cl_2 ("Synthetic Protocol 10"). All the coupling reactions were allowed to proceed overnight. The synthesis was monitored by the ninhydrin reaction, which showed close to quantitative incorporation of all residues. During the synthesis, portions of the shorter fragments H-[C(Z)aeg]$_5$-Lys(ClZ)-MBHA resin, H-[C(Z)aeg]$_6$-Lys(ClZ)-MBHA resin, H-[C(Z)aeg]$_7$-Lys(ClZ)-MBHA resin, H-[C(Z)aeg]$_8$-Lys(ClZ)-MBHA resin, and H-[C(Z)aeg]$_9$-Lys(ClZ)-MBHA resin were taken out for HF cleavage.

(b) Cleavage, Purification, and Identification of H-[Caeg]$_5$-Lys-NH$_2$.

The protected Boc-[C(Z)aeg]$_5$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 10.8 mg of crude material upon HF cleavage of 60.1 mg dry H-[C(Z)aeg]$_5$-Lys(ClZ)-MBHA resin.

(c) Cleavage, Purification, and Identification of H-[Caeg]$_6$-Lys-NH$_2$.

The protected Boc-[C(Z)aeg]$_6$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 13.4 mg of crude material upon HF cleavage of 56.2 mg dry H-[C(Z)aeg]$_6$-Lys(ClZ)-MBHA resin.

(d) Cleavage, Purificati n, and Identificati n of H-[Caeg]$_8$-Lys-NH$_2$.

The protected Boc-[C(Z)aeg]$_8$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 16.8 mg of crude material upon HF cleavage of 65.6 mg dry H-[C(Z)aeg]$_8$-Lys(ClZ)-MBHA resin.

(e) Cleavage, Purification, and Identification of H-[Caeg]$_{10}$-Lys-NH$_2$.

The protected Boc-[C(Z)aeg]$_{10}$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 142.4 mg of crude material upon HF cleavage of 441 mg dry H-[C(Z)aeg]$_{10}$-Lys(ClZ)-MBHA resin.

EXAMPLE 106

Solid-Phase Synthesis of H-[Taeg]$_2$-Caeg-[Taeg]$_2$-Caeg-[Taeg]$_4$-Lys-NH$_2$

(a) Stepwise Assembly of Boc-[Taeg]$_2$-C(Z)aeg-[Taeg]$_2$-C(Z)aeg-[Taeg]$_4$-Lys(ClZ)-MBHA Resin.

About 0.3 g of wet H-[Taeg]$_2$-C(Z)aeg-[Taeg]$_4$-Lys(ClZ)-MBHA resin from the earlier synthesis of Boc-[Taeg]$_3$-C(Z)aeg-[Taeg]$_4$-Lys(ClZ)-MBHA resin was placed in a 5 ml SPPS reaction vessel. After coupling of the next residue five times, a total incorporation of BocC(Z)aeg of 87% was obtained. The five repeated couplings were carried out with 0.18 M BocC(Z)aeg-OPfp in 2 ml of TFE/CH_2Cl_2 (1:2, v/v), 2 ml of TFE/CH_2Cl_2 (1:2, v/v), 2 ml of TFE/CH_2Cl_2 (1:2, v/v) with two drops of dioxane and two drops of DIEA (this condition gave only a few per cent coupling yield), 2 ml of TFE/CH_2Cl_2 (1:2, v/v) plus 0.5 g phenol, and 1 ml of CH_2Cl_2 plus 0.4 g of phenol, respectively. The two final Taeg residues were incorporated close to quantitatively by double couplings with 0.25 M BocTaeg-OPfp in 25% phenol/CH_2Cl_2. All couplings were allowed to proceed overnight.

(b) Cleavag , Purification, and Identificati n of H-$[Taeg]_2$-Caeg-$[Ta\ g]_2$-Ca g-$[Taeg]_4$-Lys-NH_2

The protected Boc-$[Taeg]_2$-C(Z)aeg-$[Taeg]_2$-C(Z)aeg-$[Taeg]_4$-Lys(ClZ)-MBHA resin was treated as described in Example 17c to yield about 7 mg of crude material upon HF cleavage of 80.7 mg dry H-$[Taeg]_2$-C(Z)aeg-$[Taeg]_2$-C(Z)aeg-$[Taeg]_4$-Lys(ClZ)-MBHA resin. The crude product was purified to give 1.2 mg of H-$[Taeg]_2$-Caeg-$[Taeg]_2$-Caeg-$[Taeg]_4$- Lys-NH_2 (>99.9% purity).

EXAMPLE 107

SYNTHESIS OF A PNA WITH TWO ANTI PARALLEL STRANDS TIED TOGETHER

Synthesis of H-[Taeg]-[Taeg]-[Taeg]-[Gaeg]-[Taeg]-[Taeg]-[Taeg]-[6-AHA]-[aeg]-[6-AHA]-[Taeg]-[Taeg]-[Taeg]-[Aaeg]-[Taeg]-[Taeg]-[Taeg]-LYS-NH_2. (6-AHA = 6-aminohexanoic acid) (Figure 26)

The protected PNA was assembled onto a Boc-Lys(ClZ) modified MBHA resin with a substitution of approximately 0.30 mmol/g. Capping of uncoupled amino groups was only carried out before the incorporation of the BocGaeg-OH monomer. Synthesis was initiated on 1.00 g (dry weight) of preswollen (overnight in DCM) and neutralized Boc-Lys(ClZ)-MBHA resin. The incorporation of the monomers followed the protocol of Example 32 and Example 71. The coupling reaction was monitored by qualitative ninhydrin reaction (kaiser test). In case of a positive Kaiser test, the coupling reaction was repeated until the test showed no coloration of the beads. Final deprotection, cleavage from support, and purification were performed according to standard procedures.

EXAMPLE 108

Alternative protecting group strategy for PNA-synthesis (Figure 27).

(a) Synthesis f test c mp unds.

2-amino-6-O-benzyl purine. T a solution f 2.5 g (0.109 mol) f sodium in 100 ml of benzyl alcohol was added 10.75 g (0.063 mol) of 2-amino-6-chl ropurine. The mixture

was stirred for 12 h at 120 0°C. The solution was cooled to room temperature and neutraliz d with ac tic acid and extracted with 10 portions of 50 ml of 0.2 N sodium hydroxide. The collected sodium hydroxide phases were washed with 100 ml of diethyl ether and neutralized with acetic acid, whereby precipitation starts. The solution was cooled to 0°C and the yellow precipitate was collected by filtration. Recrystallization from ethanol gave 14.2 g 92% of pure white crystals of the target compound. 1H-NMR (250 MHz--DMSO-d6) d ppm: 8-H, 7.92; benzyl aromatic, 7.60-7.40; $2NH_2$, 6.36; benzyl CH2, 5.57.

(2-amino-6-O-benzyl purinyl)methylethanoate. A mixture of 5 g (0.0207 mol) of 2-amino-6-O-benzyl-purine, 30 ml of DMF and 2.9 g (0.021 mol) of potassium carbonate was stirred at room temperature. Methyl bromoacetate (3.2 g; 1.9 ml; 0,0209 mol) was added dropwise. The solution was filtrated after 4 h and the solvent was removed under reduced pressure (4 mmHg, 40°C). The residue was recrystallized two times from ethyl acetate to give 3.7 g (57%) of the target compound. 1H-NMR (250 MHz, DMSO-d6) d ppm: 8-H, 7.93; benzyl aromatic 7.4-7.6; $2-NH_2$, 6.61; benzyl CH2, 5.03; CH2, 5.59; OCH3, 3.78.

(2N-p-Toluene sulfonamido-6-O-benzyl purinyl) methyl ethanoate. To a solution of 0.5 g (1.6 mmol) of (2-amino-6-O-benzyl purinyl) methyl ethanoate in 25 ml methylene chloride was added 0.53 g (1.62 mmol) of p-toluenesulfonic anhydride and 0.22 g (1.62 mmol) of potassium carbonate. The mixture was stirred at room temperature. The mixture was filtered and the solvent was removed at reduced pressure (15 mmHg, 40°C). Diethyl ether was added to the oily residue. The resulting solution was stirred overnight, whereby the target compound (0.415 mg; 55%) precipitated and was collected by filtration. 1H-NMR (250 MHz, DMSO-d6) d ppm: 8-H, 8.97; aromatic 7.2-7.8; benzyl CH2, 5,01; CH2, 4.24; OCH3, 3.73; CH3, 2.43.

(b) Stability f the t syl protect d base-residue in TFA and HF.

The material was subjected t the standard deprotecti n conditions (TFA-depr tection) and the final cleavage

conditions with HF. The pr ducts were then subjected to HPLC-analysis using a 4 μ RCM 8x10 Nova pack column and s lvents A (0.1% TFA in water) and B (0.1% TFA in acetonitrile) according to the following time gradient with a flow of 2 ml/min.

Time	% A	%B
0	100	0
5	100	0
35	0	100
37	0	100
39	100	0

The following retention times were found: (a) Compound 1: 30.77 min; (b) compound 2: 24.22 min; and (c) compound 3: 11.75 min. The analysis showed that the O6-benzyl group was removed both by TFA and HF, whereas there was no cleavage of the tosyl group in TFA, but quantitative removal in HF under the standard cleavage conditions.

EXAMPLE 109

5-Bromouracil-N^1-methyl acetate

5-Bromouracil (5.00 g; 26.2 mmol) and potassium carbonate (7.23 g; 52.3 mmol) were suspended in DMF (75 ml). Methyl bromoacetate (2.48 ml; 26.1 mmol) was added over a period of 5 min. The suspension was stirred for 2 h at room temperature, and then filtered. The solid residue was washed twice with DMF, and the combined filtrates were evaporated to dryness, *in vacuo*. The residue was an oil containing the title compound, DMF and some unidentified impurities. It is not necessary to purify the title compound before hydrolysis. ^{1}H-NMR (DMSO-d_6, 250 MHz); 8.55 (impurity); 8.27 (CBr=C$\underline{H}$N); 8.02 (impurity); 4.76 (impurity); 4.70 (impurity); 4.62 (NC$\underline{H}_2$COOCH$_3$); 3.78 (COOC$\underline{H}_3$); 2.96 (DMF); 2.80 (DMF). ^{13}C-NMR (DMSO-d_6, 250 MHz); 168.8 ($\underline{C}$OOCH$_3$); 172.5 (CH=CBr$\underline{C}$ON); 161.6 (DMF); 151.9 (N$\underline{C}$ON); 145.0 (CO-CBr=$\underline{C}$HN); 95.6 (CO$\underline{C}$Br=CHN); 52.6 (impurity); 52.5 (O$\underline{C}$H$_3$); 49.7 (impurity); 48.8 (N$\underline{C}$H$_2$COOMe); 43.0 (impurity); 36.0 (DMF). UV(Methanol; $_{max}$nm); 226; 278. IR (KBr;cm^{-1}_; 3158s (_NH); 1743vs (_C=O, COOMe); 1701vs (_C=O, CONH); 1438vs (∂ CH, CH$_3$O); 1223vs (_ C-O,

COOMe); 864 m (∂ CH, Br=C-H). FAB-MS m/z (assignment): 265/263 (M+H).

EXAMPLE 110

(5-Bromouracil)acetic acid

Water (30 ml) was added to the oil of the crude product from Example 109 and the mixture was dissolved by adding sodium hydroxide (2M, 60 ml). After stirring at 0°C for 10 min, hydrochloric acid (4M, 45 ml) was added to pH=2 and the title compound precipitated. After 50 min, the solid residue was isolated by filtration, washed once with cold water, and then dried *in vacuo* over sicapent. Yield: 2.46 g (38%). Mp, 250°-251°C. Anal. for $C_6H_5BrN_2O_4$. Found (calc.): C: 28.78 (28.94); H: 2.00 (2.02); Br: 32.18 (32.09); N: 11.29 (11.25). ^{1}H-NMR (DMSO-d_6, 250 MHz): 12,55 (1H.s,COO<u>H</u>); 11.97 (1H,s,N<u>H</u>); 8.30 (1H,s,C=C-<u>H</u>); 4.49 (2H,s,NC<u>H</u>$_2$COOH). ^{13}C-NMR (DMSO-d_6, 250 MHz); 169.4 (<u>C</u>OOH); 159.8 (NH<u>C</u>OCBr=CH); 150.04 (N<u>C</u>ON); 145.8 (COCBr=<u>C</u>HN); 94.6 (CO<u>C</u>Br=CHN); 48.8 (N<u>C</u>H$_2$COOH). UV (Methanol; $_{max}$nm); 226; 278. IR (KBr; cm^{-1}); 3187s (_NH); 1708vs (_C=O,COOH); 1687vs; 1654VS (_C=O, CONH); 1192s (_C-O, COOH); 842 m (∂ CH, Br-C=C-H). FAB-MS m/z (assignment, relative intensity); 251/249 (M + H,5).

EXAMPLE 111

N-(Boc-aminoethyl)-N-(5-bromouracil)methylenecarbonoylglycine ethyl ester

Boc-aminoethylglycine ethyl ester (1.80 g; 7.30 mmol) was dissolved in DMF (10 ml). Dhbt-OH (1.31 g; 8.03 mmol) was added, whereby a precipitate was formed. DMF (2 x 10 ml) was added until the precipitate was dissolved. The product of Example 110 (2.00 g; 8.03 mmol) was added slowly to avoid precipitation. Methylene chloride (30 ml) was added, and the mixture was cooled to 0°C and then filtered. The precipitate, DCU, was washed twice with methylene chloride. To th c mbined filtrat was added methylene chloride (100 ml). The mixture was washed with half saturat d $NaHCO_3$-soluti n (3 x 100 ml, H_2O:saturated $NaHCO_3$-solution 1:1 v/v), then with

dilute $KHSO_4$-s luti n (2 x 100 ml, H_2O:saturated $KHSO_4$-solution 4:1 v/v), and finally with saturated NaCl-s luti n (1 x 100 ml). The organic phase was dried over magnesium sulphate, filtered, and evaporated to dryness *in vacuo* (about 15 mmHg and then about 1 mmHg). The résidue was suspended in methylene chloride (35 ml), stirred for 45 min at room temperature, and filtered (the precipitate was DCU). Petroleum ether (2 volumes) was added dropwise to the filtrate at 0°C, whereby an oil precipitated. The liquor was decanted and the remaining oil dissolved in methylene chloride (20-50 ml). Precipitated was effected by the addition of petroleum ether (2 volumes). This procedure was repeated 5 times until an impurity was removed. The impurity can be seen at TLC with 10% $MeOH/CH_2Cl_2$ as the developing solvent. The resulting oil was dissolved in methylene chloride (25 ml) and evaporated to dryness *in vacuo*, which caused solidification of the title compound. Yield: 2.03 g ((58%). Mp. 87°-90°C. Anal. for $C_{17}H_{25}BrN_4O_7$. Found (calc.): C: 42.33 (42.78); H: 5.15 (5.28); Br: 17.20 (16.74); N: 1.69 (11.74). ^{1}H-NMR (DMSO-d_6, 250 MHz, J in Hz): 1.93 & 11.92 (1H,s,C=ON$\underline{H}$C=O); 8.09 & 8.07 (1H,s,C=C-$\underline{H}$); 7.00 & 6.80 (1H,t,b,BocN$\underline{H}$); 4.80 & 4.62 (2H,s,NC$\underline{H}_2$CON); 4.35 & 4.24 (2H,s,NC$\underline{H}_2$COOEt); 4.27-4.15 (2H,m's, COOC$\underline{H}_2CH_3$0); 3.47-3.43 (2H,m's, BocNHCH_2C$\underline{H}_2$N); 3.28-3.25 & 3.12-3.09 (2H,m's,BocNHC$\underline{H}_2$CH-$_2$N): 1.46 & 1.45 (9H,s,^{t}Bu); 1.26 & 1.32 (3H,t,J=7.1, COOCH_2C$\underline{H}_3$). ^{13}C-NMR (DMSO-d_6, 250 MHz); 169.3 & 169.0 (^{t}BuO$\underline{C}$=O); 167.4 & 167.1 ($\underline{C}$OOEt); 159.8 (C=C-$\underline{C}$ON); 155.9 (NCH$_2\underline{C}$ON); 150.4 (N$\underline{C}$ON); 145.9 (COCBr-$\underline{C}$HN); 94.5 (CO$\underline{C}$Br=CHN); 78.2 (Me$_3\underline{C}$); 61.3 & 60.7 (CO$\underline{C}H_2CH_3$); 49.1 & 48.0 (N$\underline{C}H_2$COOH); 48.0 & 47.0 (N$\underline{C}H_2$CON); 38.6 (BocNHCH$_2\underline{C}H_2$N); 38.2 (BocNH$\underline{C}H_2CH_2$N); 26.3 (C($\underline{C}H_3$)$_3$); 14.1 (COCH$_2\underline{C}H_3$). UV (Methanol; $_{max}$ NM): 226; 280. IR (KBr, CM^{-1}): 3200ms, broad (_NH); 168vs, vbroad (_C=O, COOH, CONH); 1250s (_ C-O, COOEt); 1170s (_C-O, COO^{t}Bu); 859m (∂ CH, Br-C=C-H). FAB-MS m/z (assignm nt, relative intensity): 479/477 (M + H, 5); 423/421 (M + 2H - ^{t}Bu, 8); 379/377 (M + 2H - B c, 100); 233/231 (M - backbone, 20).

EXAMPLE 112

N-(B -amin thyl)-N-(5-brom uracyl-N^1-methyleneoarb noyl)-glycine

The product of Example 111 (1.96 g; 4.11 mmol) was dissolved in methanol (30 ml) by heating, and then cooled t 0°C. Sodium hydroxide (2M, 30 ml) was added, and the mixture stirred for 30 min. HCl (1M, 70 ml) was added to pH = 2.0. The water phase was extracted with ethyl acetate (3 x 65 ml + 7 x 40 ml). The combined ethyl acetate extractions wer washed with saturated NaCl-solution (500 ml). The ethyl acetate phase was dried over magnesium sulphate, filtered and evaporated to dryness *in vacuo*. Yield: 1.77 g (96%). Mp. 92°-97°C. Anal. for $C_{15}H_{21}BrN_4O_7$. Found (calc.): C: 40.79 (40.10); H: 5.15 (4.71); Br: 14.64 (17.70); N: 11.35 (12.47). ^{1}H-NMR (DMSO-d_6, 250 MHz, J in Hz): 12.83 (1H,s,COO$\underline{H}$); 11.93 & 11.91 (1H,s,C=ON$\underline{H}$C=O); 8.10 & 8.07 (1H,s,C=C-$\underline{H}$); 7.00 & 6.81 (1H,t,b,BocN$\underline{H}$); 4.79 & 4.61 (2H,s,NC$\underline{H}_2$CON); 4.37 & 4.25 (2H,s,NC$\underline{H}_2$COOH); 3.46-3.39 (2H,m's, BocNHCH$_2$C$\underline{H}_2$N); 3.26-3.23 & 3.12-3.09 (2H,m's, BocNHC$\underline{H}_2$CH$_2$N); 1.46 (9H,s,^{t}Bu). ^{13}C-NMR 9DMSO-d_6,250 MHz); 170.4 (^{t}BuO$\underline{C}$=O); 166.9($\underline{C}$OOH); 159.7 (C=C-$\underline{C}$ON); 155.8 (NCH$_2\underline{C}$ON); 150.4 (N$\underline{C}$ON); 145.9 (COCBr=$\underline{C}$HN); 94.4 (CO$\underline{C}$Br=CHN); 78.1 (Me$_3\underline{C}$); 49.1 & 48.0 (N$\underline{C}$H$_2$COOH); 47.7 & 47.8 (N$\underline{C}$H$_2$CON); 38.6 (BocNHC$_2\underline{C}$H$_2$N); 38.1 (Boc NH$\underline{C}$H$_2$CH$_2$N); 28.2 (C($\underline{C}$H$_3$)$_3$). UV (Methanol; $_{max}$nm); 226; 278. IR (KBr,cm^{-1}): 3194ms, broad (_NH); 1686vs, vbroad (_C=O COOH, CONH); 1250s (_C-O,COOH); 1170s (_C-O,COO^{t}Bu); 863m (∂ CH, Br-C=C-H). FAB-MS m/z (assignment, relative intensity): 449/451 (M + H, 70); 349/351 (M + 2H -Boc, 100); 231/233 (M - backbone, 20).

EXAMPLE 113

Uracil-N^1-methyl acetate

Uracil (10.0 g; 89.2 mmol) and potassium carbonate (24.7 g; 178 mmol) were suspended in DMF (250 ml). Methyl bromoacetate (8.45 ml; 89.2 mmol) was added over a period of 5 min. The suspension was stirred vernight under nitrogen at room temperature, and th n filtered. TLC (10% methan l in ethylene chl ride) indicated incomplete c nversion of uracil.

The solid residue was washed twic with DMF, and the combined filtrates were evap rated to dryn ss *in vacuo*. The precipitate was suspended in water (60 ml) and HCl (2.5 ml, 4M) was added to pH = 2. The suspension was stirred for 30 min at 0°C, and then filtered. The precipitated title compound was washed with water and dried, *in vacuo*, over sicapent. Yield: 9.91 g (60%). Mp. 182° - 183°C. Anal. for $C_6H_8N_2O_4$. Found (calc.): C: 45.38 (45.66); H: 4.29 (4.38); N: 15.00 (15.21). ^{1}H-NMR (DMSO-d_6, 250 MHz, J in Hz): 1.47 (1H,s, N<u>H</u>); 7.68 (1H,d,$J_{H-C=C-H}$=7.9), CH=C<u>H</u>N); 5.69 (1H,d,$J_{H-C=C-H}$=7.9), C<u>H</u>=CHN); 4.59 (2H,s,NCH_2COOMe); 3.76 (3H,s,COOCH_3). ^{13}C-NMR (DMSO-d_6, 250 MHz); 168.8 (<u>C</u>OOMe); 164.0 (C=C-<u>C</u>ON); 151.1 (N<u>C</u>ON); 146.1 (COCH=<u>C</u>HN); 101.3 (CO<u>C</u>H=CHN); 52.5 (COO<u>C</u>H_3); 48.7 (N<u>C</u>H_2COOMe). UV (Methanol; $_{max}$nm): 226; 261. IR (KBr; cm^{-1}); 3164s (_NH); 1748vs (_C=O, COOMe); 1733vs (_C=O, CONH); 1450vs (∂ CH, CH_3O); 1243VS (_C-O,COOMe); 701m (∂ CH, H-C=C-H). FAB-MS m/z (assignment); 185 (M+H).

EXAMPLE 114

Uracilacetic acid

Water (90 ml) was added to the product of Example 113 (8.76 g; 47.5 mmol), followed by sodium hydroxide (2M, 40 ml). The mixture was heated for 40 min, until all the methyl ester has reacted. After stirring at 0°C for 15 min, hydrochloric acid (4M, 25 ml) was added to pH=2. The title compound precipitated and the mixture was filtered after 2-3 h. The precipitate was washed once with the mother liquor and twice with cold water and dried *in vacuo* over sicapent. Yield: 6,.66 g (82%). Mp. 288°-289°C. Anal. for $C_6H_6N_2O_4$. Found (calc.): C: 42.10 (42.36), H: 3.43 (3.55); N: 16.25 (16.47)/ ^{1}H-NMR (DMSO-d_6), 250 MHz, J in Hz): 13.19 (1H,s,COO<u>H</u>); 11.41 (1H,s,N<u>H</u>); 7.69 (1H,d,$J_{H-C=C-H}$=7.8, $J_{H-C-C-N-H}$=2.0, coc<u>h</u>=chn); 4.49 (2H,s,NCH_2COOH). ^{13}C-NMR (DMSO-d_6, 2509 MHz); 169.9 (<u>C</u>OOH); 163.9 (CH=CH<u>C</u>ON); 151.1 (N<u>C</u>ON); 146.1 (COCH=<u>C</u>HN); 100.9 (CO<u>C</u>H=CHN); 48.7 N<u>C</u>H_2COOH. UV (Methanol; $_{max}$nm): 246; 263. IR (KBr; cm^{-1}): 3122s (_NH); 1703vs (_C=O, COOH); 1698vs,

1692vs (_C=O, CONH); 1205s (_C-O,COOH); 676 (∂ CH, H-C=C-H). FAB-MS m/z (assignment): 171 (M + H).

EXAMPLE 115

N-(Bocaminoethyl)-N-(uracil-N^1-methylenecarbonoyl)glycine ethyl ester

(Bocaminoethyl)glycine ethyl ester (2.00 g; 8.12 mmol) was dissolved in DMF (10 ml). Dhbt-OH (1.46 g; 8.93 mmol) was added and a precipitate was formed. DMF (2 x 10 ml) was added until all was dissolved. The product of Example 114 (1.52 g; 8.93 mmol) was added slowly to avoid precipitation. Methylene chloride (30 ml) was added, and the mixture was cooled to 0°C, whereafter DDC (2.01g; 9.74 mmol) was added. The mixture was stirred for 1 h at 0°C, at 2 h at room temperature, and then filtered. The precipitated DCU was washed twice with methylene chloride. To combined filtrate was added methylene chloride (100 ml), and the solution washed with half-saturated NaHCO3-solution (3 x 100 ml, H_2O:saturated $NaHCO_3$-solution 1:1 v/v), then with dilute $KHSO_4$.solution (2 x 100 ml, H_2O:saturated $KHSO_4$-solution 4:1 v/v) and finally with saturated NaCl-solution (1 x 100 ml). The organic phase was dried over magnesium sulphate, filtered and evaporated to dryness in vacuo (about 15 mmHg and then about 1mmHg). Th residue was suspended in methylene chloride (32 ml), and stirred for 35 min at room temperature, and 30 min at 0°C, and then filtered. The precipitate (DCU) was washed with methylene chloride. Petroleum ether (2 volumes) was added dropwise to the combined filtrate at 0°C, which caused separation of an oil. The mixture was decanted, the remaining oil was then dissolved in methylene chloride (20 ml), and then again precipitated by addition of petroleum ether (2 volumes). This procedure was repeated 5 times until an impurity was removed. The impurity can be seen by TLC with 10% MeOH/CH_2Cl_2 as the developing solvent. The resulting oil was dissolved in methylene chloride (20 ml) and evaporated to dryness *in vacuo*, which caused s lidification of the title compound. Yield: 1.71 g (53%). Mp. 68.5° - 75.7°C. Anal for $C_{17}H_{26}N_4O_7$.

Found (calc.): C: 50.61 (51.25); H: 6.48 (6.58); N: 13.33 (14.06). ^{1}H-NMR (DMSO-d_6,250 MHz,J in Hz): 11.36 (1H,s,C=ONHC=O); 7.51 & 7.47 (1H,d,$J_{H-C=C-H}$+ 6.1; COCH=X-H); 7.00 & 6.80 (1H,t,b, BocNH); 5.83 & 5.66 (1H,d,$J_{H-C=C-H}$= 5.7, COCH=CH); 4.78 & 4.60 (2H,s,NCH_2CON); 4.37 & 4.12 (2H,s,NCh_2COOEt); 4.30 - 4.15 (2H,m's,$COOCH_2CH_3$); 3.49-3.46 (2H,m's, $BocNHCH_2CH_2n$); 3.27 3.23 & 3.11-3.09 (2H, m's, $BocNHCH_2CH_2N$; 1.46 (9H, s, ^{t}Bu); 1.39-1.23 (3H, m's, $COOCH_2CH_3$). ^{13}C-NMR (DMSO-d_6, 250 MHz): 169.4 & 169.0 (^{t}BuOC=O); 167.6 & 167.3 (COOEt); 163.8 (CH=CHCON); 155.8 (NCH_2CON); 151.0 (NCON); 146.3 (COCH=CHN); 100.8 (COCH=CHN); 78.1 (Me_3C); 61.2 & 60.6 ($COOCH_2CH_3$); 49.1 (NCH_2COOEt); 47.8 & 47.0 (NCH_2CON); 38.6 ($BocNHCH_2CH_2N$); 38.1 & 37.7 ($BocNHCH_2N$); 28.2 ($C(CH_3)_3$); 14.1 ($CO-OCH_2CH_3$. UV (Methanol; $_{max}$ nm); 226; 264. IR (KBr; cm^{-1}): 3053m (_NH); 1685vs, vbroad (_C=O, COOH, CONH); 1253s (_C-O, COOEt); 1172s (_C-O, COO^{t}Bu); 718w (∂ CH, C-C-C-H), FAB-MS m/z (assignment, relative intensity); 399 (M + H, 35); 343 (M + 2H - ^{t}Bu, 100); 299 (M + 2H - Boc, 100); 153 (M-backbone, 30).

EXAMPLE 116

N-(Bocaminoethyl)-N-(uracilmethylenecarbonoyl)glycine

The product of Example 115 (1.56 g; 3.91 mmol) was dissolved in methanol (20 ml) and then cooled to 0°C. Sodium hydroxide (2M, 20 ml) was added, and the mixture was stirred for 75 min at 0°C. Hydrochloric acid (1M, 46 ml) was added to pH = 2.0. The water phase was extracted was ethyl acetate (3 X 50 ml + 7 x 30 ml). The combined ethyl acetate extractions were washed with saturated NaCl solution (360 ml). The ethyl acetate phase was dried over magnesium sulphate, filtered, and evaporated to dryness, *in vacuo*. The residue was dissolved in methanol and evaporated to dryness, *in vacuo*. Yield: 0.55 g (38%). Mp 164° - 170°C. Anal. for $C_{15}H_{22}N_4O_7$. F und (calc.): C: 46.68 (48.65); H: 6.03 (5.99); N: 1461 (15.13). ^{1}H-NMR (DMSO-d_6, 250 MHz, J in Hz); 12.83 (1H, s, COOH); 11.36 (1H, s, C=ONHC=O); 7.52-7.45 (1H, m's, COCH=CHN); 7.00 & 6.82 (1H, t,b, BocNH); 5.67-5.62 (1H, M's, COCH=CHN);

4.76 & 4.58 (2H, s, NCH_2CON); 4.26 & 4.05 (2H, s, $NC\underline{H}_2COOH$): 3.46-3.39 (2H, m's, $BocNHCH_2C\underline{H}_2N$); 3.25-3.23 & 3.15-3.09 (2H, m's, $BocNHC\underline{H}_2CH_2N$); 1.46 (9H, s, ^tBu). ^{13}C-NMR (DMSO-d_6, 250 MHz); 170.5 ($^tBuO\underline{C}$=O); 167.2 (COOH); 163.9 (C=C-$\underline{C}$ON); 155.8 ($NCH_2\underline{C}ON$); 151.1 (N$\underline{C}$ON); 146.4 (COCH=$\underline{C}$HN); 100.8 (CO$\underline{C}$H=CHN); 78.1 ($Me_3\underline{C}$); 49.1 & 47.8 ($N\underline{C}H_2$ COOH); 47.6 & 46.9 ($N\underline{C}H_2CON$); 38.6 ($BocNHCH_2\underline{C}H_2N$); 38.1 & 37.6 ($BocNH\underline{C}H_2CH_2N$); 28.2 ($C(\underline{C}H_3)_3$). UV (Methanol; λ_{max} nm); 226; 264. IR (KBr; cm^{-1}); 3190 (_NH); 1685vs, vbroad (_C=O, COOH, CONH); 1253s (_C-O, COOH); 1171s (_C-O, COO^tBU); 682w (∂ CH, H-C=C-H). FAB-MS m/z (assignment, relative intensity): 371 (M + H, 25); 271 (M + H -Boc, 100).

EXAMPLE 117

H-U10-LysNH$_2$

Synthesis of the title compound was accomplished by using "Synthetic Protocol 10". The synthesis was initiated on approximately 100 mg Lys (ClZ)-MHBA-resin. The crude product (12 mg) was pure enough for hybridization studies. The hybrid between 5'-(dA)10 and H-U10 had *Tm* of 67.5°C.

EXAMPLE 118

Deprotection and Cleavage of H-$[Cacg]_{10}$-Lys-NH_2 by Trifluoromethansulfonic Acid (TFMSA). An Alternative Method to Deprotection and Cleavage by Hydrogen Fluoride (HF).

(a) Deprotection of Side-Chain Protecting Groups by a "Low-Acidity" TFMSA-TFA-DMS Procedure

A portion of ca. 0.4 g wet Boc-$[Cacg]_{10}$-Lys(ClZ)-MBHA resin (prepared in one of the previous examples) was placed in a 5 ml solid-phase reaction vessel. The n-Terminal Boc group was removed by the following protocol: (1) 50% TFA/CH_2Cl_2, 2 x 1 min and 1 x 30 min; (2) 100% TFA, 2 x 1 min and drain. In order to deprotect the benzyl-based side-chain protecting groups a so-called "low-acidity" TFMSA procedure was carried out as follows: A stock solution (a) containing 5 ml of TFA-DMS-m-cresol (2:6:2, v/v/v) and a stock soluti n (B) c ntaining TFA-TFMSA (8:2, v/v) were pr par d. Next, the f llowing steps w re carri d out: (3) 1 ml of stock solution (A) is add d to the PNA-r sin in th r action vessel with shaking for 2 min. N drain; (4) 1 ml of stock solution (B) (cool d with ic /water) is add d in porti ns of 200 µl ev ry

10th minute ov r a period f 40 min, and shaking is continued for another 50 min; (5) drain and washing with 100% TFA, 5 x 1 min. and drain.

(b) Cleavage from the Resin by a "High-Acidity" TFMSA-TFA Procedure

In order to cleave the above-mentioned deprotected PNA from the resin a so-called "high acidity" TFMSA procedure was carried out as follows: A stock solution (C) containing m-cresol-TFA (2:8, v/v) was prepared. Next, the following steps were carried out: (6) 1 ml of stock solution (C) was added to the deprotected PNA-resin in the SPPS vessel with shaking for 2 min; (7) 1 ml of stock solution (B) (cooled with ice/water) is added in portions of 200 µl over a period of 30 min and shaking is continued for another 150 min; (5) the 2 ml solutin in the reaction vessel is "blown out" through the filter into a 20 ml solution of diethylether cooled with dry ice/isopropanol. In order to complete the precipitation process, 200 µl of anhydrous pyridine is added dropwise to th acid-ether mixture; (8) centrifugalization at 3000 rpm for 5 min; (9) the supernatant is decanted and the precipitate is washed three times with cold diethylether, dried, dissolved in water, and lyophilized.

(c) Purification and Identification of H-$[Caeg]_{10}$-Lys-NH_2

An analytical HPLC chromatogram showed a nice crude product of good purity and a profile almost identical to that obtained from the HF cleavage of H-$[Caeg]_{10}$-Lys-NH_2, except that an additional peak, of course, arising from pyridin TFMSA salt elutes early in the chromatogram. Purification and identification was carried out by the usual procedures.

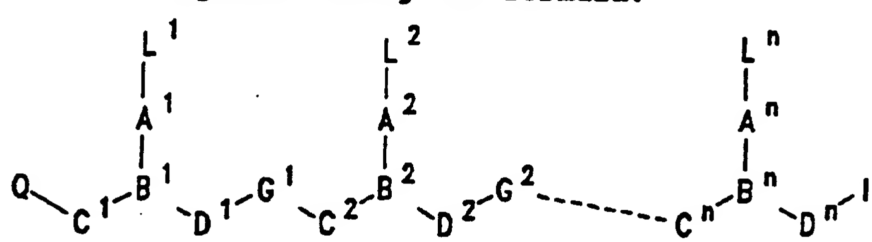
Those skilled in the art will appreciate that numerous changes and modifications may be made to the preferr d embodiments of the invention and that such changes and modifications may be made without departing fr m th spirit of the invention. It is therefore intended that the appended claims cover all such quival nt variations as fall within the true spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. A compound comprising a polyamide backbone bearing a plurality of ligands that are individually bound to aza nitrogen atoms located within said backbone, at least one of said ligands being a naturally occurring nucleobase, a n n-naturally occurring nucleobase, a DNA intercalator, or a nucleobase-binding group.

2. The compound of claim 1 wherein said aza nitrogen atoms are separated from one another in said backbone by from 4 to 6 intervening atoms.

3. A compound having the formula:

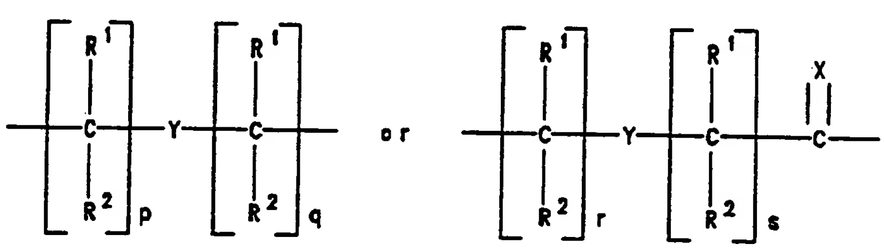


wherein:

n is at least 2,

each of L^1-L^n is independently selected from the group consisting of hydrogen, hydroxy, $(C_1$-$C_4)$alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, and reporter ligands, at least one of L^1-L^n being a naturally occurring nucleobase, a non-naturally occurring nucleobase, a DNA intercalator, or a nucleobase-binding group;

each of A^1-A^n is a single bond, a methylene group or a group of formula:



where:

X is O, S, S , NR^3, CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR^4;

each of p and q is zero or an integer from 1 to 5, the sum p+q being not more than 10;

each of r and s is zero or an integer from 1 t 5, the sum r+s being not more than 10;

each R^1 and R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl which may be hydroxy- or alkoxy- or alkylthio-substituted, hydroxy, alkoxy, alkylthio, amino and halogen; and

each R^3 and R^4 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl, hydroxy- r alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio and amino;

each of B^1-B^n is N or R^3N^+, where R^3 is as defined above;

each of C^1-C^n is CR^6R^7, CHR^6CHR^7 or $CR^6R^7CH_2$, where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_2-C_6)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6)alkoxy, (C_1-C_6)alkylthio, NR^3R^4 and SR^5, where R^3 and R^4 are as defined above, and R^5 is hydrogen, (C_1-C_6)alkyl, hydroxy-, alkoxy-, or alkylthio- substituted (C_1-C_6)alkyl, r R^6 and R^7 taken together complete an alicyclic or heterocyclic system;

each of D^1-D^n is CR^6R^7, $CH_2CR^6R^7$ or CHR^6CHR^7, where R^6 and R^7 are as defined above;

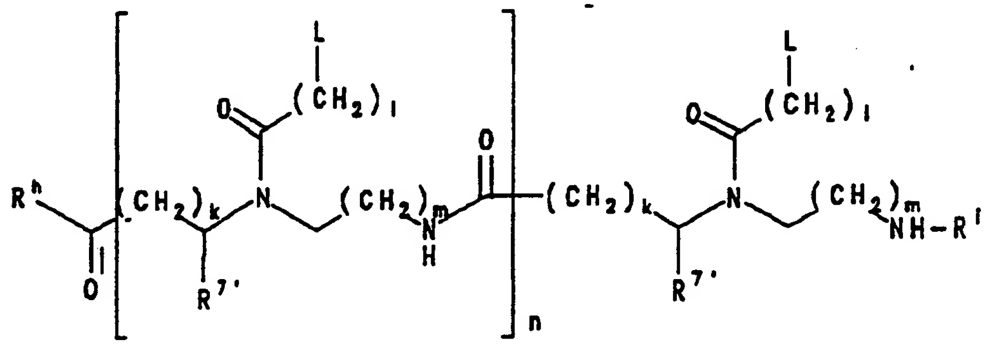
each of G^1-G^{n-1} is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO_2-$, in either orientation, where R^3 is as defined above;

Q is $-CO_2H$, $-CONR'R''$, $-SO_3H$ or $-SO_2NR'R''$ or an activated derivative of $-CO_2H$ or $-SO_3H$; and

I is $-NHR'''R''''$ or $-NR'''C(O)R''''$, where R′, R″, R‴ and R'''' are independently select d from the gr up c nsisting f hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides,

proteins, carbohydrates, lipids, steroids, olig nucleotides and soluble and non-s luble p lymers.

4. The compound of claim 3 having the formula:



wherein:

each L is independently selected from the group consisting of hydrogen, phenyl, heterocyclic moieties, naturally occurring nucleobases, and non-naturally occurring nucleobases;

each $R^{7'}$ is independently selected from the group consisting of hydrogen and the side chains of naturally occurring alpha amino acids;

n is an integer from 1 to 60,

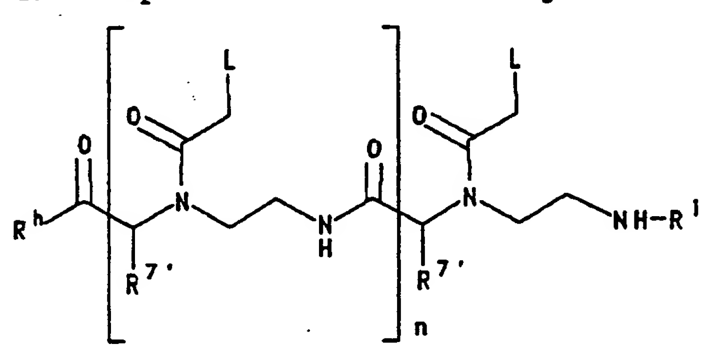
each k and m is, independently, zero or 1;

each l is zero or an integer from 1 to 5;

R^h is OH, NH_2 or $-NHLysNH_2$; and

R^i is H or $COCH_3$.

5. The compound of claim 4 having formula:

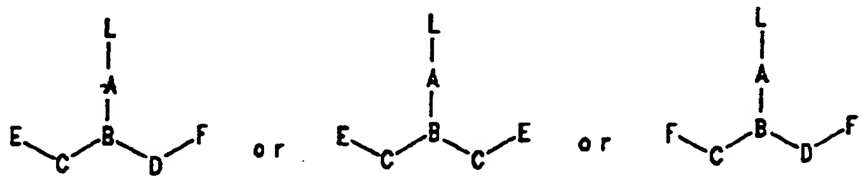


each L is independently selected from the group c nsisting of the nucle bas s thymine, adenine, cytosine, guanine, and uracil;

each $R^{7'}$ is hydrogen; and

n is an integer from 1 to 30.

6. A compound having one of the following formulas:



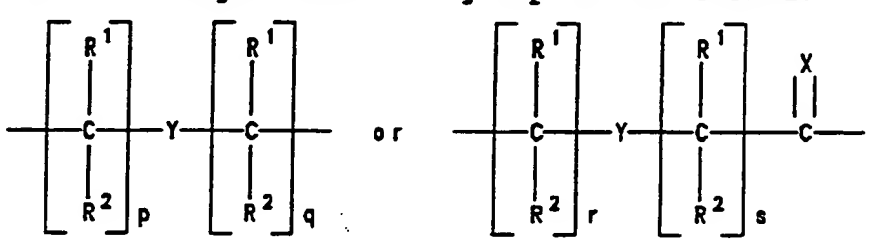
wherein:

L is selected from the group consisting of hydrogen, hydroxy, (C_1-C_4)alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, and heterocyclic moieties, reporter ligands, wherein:

at least one of L^1-L^n is a naturally occurring nucleobase, a non-naturally occurring nucleobase, a DNA intercalator, or a nucleobase-binding group; and

amino groups are, optionally, protected by amino protecting groups;

A is a single bond or a group of the formula:



where:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR^4;

each of p and q is zer r an integer from 1 to 5, the sum p+q being not m re than 10;

each of r and s is zero r an integ r from 1 to 5, the sum r+s being n t more than 10;

each R^1 and R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio, amino and halogen; and

each R^3 and R^4 is independently selected from th group consisting of hydrogen, (C_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio and amino;

B is N or R^3N^+, where R^3 is as defined above;

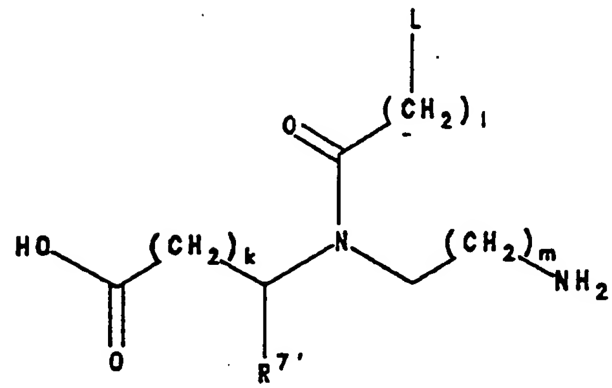
each C is CR^6R^7, CHR^6CHR^7 or $CR^6R^7CH_2$, where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_2-C_6)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6)alkoxy, (C_1-C_6)alkylthio, NR^3R^4 and SR^5, where R^3 and R^4 are as defined above, and R^5 is hydrogen or (C_1-C_6)alkyl, hydroxy-, alkoxy-, or alkylthio- substituted (C_1-C_6)alkyl, or R^6 and R^7 taken together complete an alicyclic or heterocyclic system;

each D is CR^6R^7, $CH_2CR^6R^7$ or CHR^6CHR^7, where R^6 and R^7 are as defined above;

each E is COOH, CSOH, SOOH, SO_2OH or an activated or protected derivative thereof; and

each F is NHR^3 or $NPgR^3$, where R^3 is as defined above, and Pg is an amino protecting group.

7. The comp und f claim 6 having the formula:



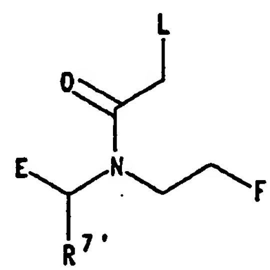
wherein:

each L is independently selected from the group consisting of hydrogen, phenyl, heterocyclic moieties, naturally occurring nucleobases, and non-naturally occurring nucleobases;

each $R^{7'}$ is independently selected from the group consisting of hydrogen and the side chains of naturally occurring alpha amino acids; and

each k, l, and m is, independently, zero or an integer from 1 to 5.

8. The compound of claim 7 having formula:



wherein:

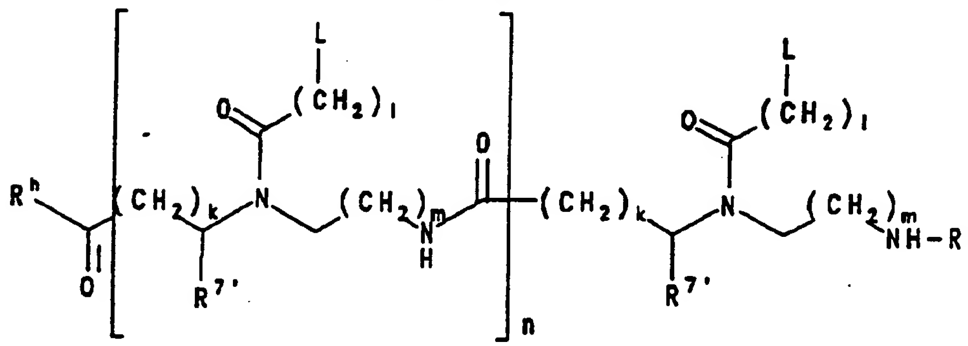
L is selected from the group consisting of the nucleobases thymine, adenine, cytosine, guanine, uracil, 5-methylcyt sine, 6-thioguanine and 5-bromouracil, and pr tected derivatives thereof;

$R^{7'}$ is hydr gen;

E is COOH r an activated r protected derivative there f; and

F is NH_2 or NHPg, where Pg is an amino protecting group.

9. A compound having the formula:



wherein:

each L is independently selected from the group consisting of hydrogen, phenyl, heterocyclic moieties, naturally occurring nucleobases, and non-naturally occurring nucleobases;

each $R^{7'}$ is independently selected from the group consisting of hydrogen and the side chains of naturally occurring alpha amino acids;

n is an integer from 1 to 60,

each k, l, and m is, independently, zero or an integer from 1 to 5;

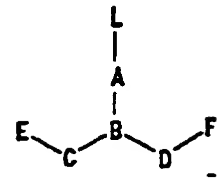
R^h is OH, NH_2 or $-NHLysNH_2$; and

R^i is H or $COCH_3$.

10. A process for preparing a compound according to claim 1, comprising the steps of:

A) providing a polymer substrate, said polymer being functionalized with a chemical group capable of forming an anchoring linkage with an amino acid;

B) coupling said polymer with a first amino acid through said anchoring linkage, said first amino acid having f rmula (IV):

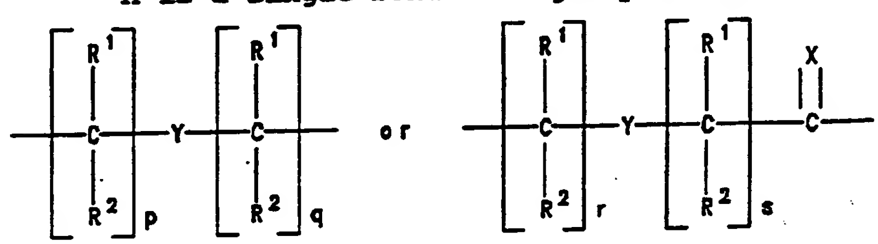


(IV)

wherein:

L is selected from the group consisting of naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, and reporter ligands, wherein amino groups are, optionally, protected by amino protecting groups;

A is a single bond or a group of the formula:



where:

X is O, S, Se, NR^3, CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR^4;

p and q are zero or integers from 1 to 5, the sum p+q being not more than 10;

r and s are zero or integers from 1 to 5, the sum r+s being not more than 10;

R^1 and R^2 are independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alkoxy, alkylthio, amino and halogen; and

R^3 and R^4 are independently selected from the group consisting of hydrogen, (C_1-C_4)alkyl, hydroxy- r alk xy- or alkylthio-substituted (C_1-C_4)alkyl, hydroxy, alk xy, alkylthio and amino;

B is N r R^3N^+, where R^3 is as defined above;

C is CR^6R^7, CHR^6CHR^7 or $CR^6R^7CH_2$, where R^6 is hydrogen and R^7 is selected from the gr up consisting

of the side chains of naturally occurring alpha amino acids, r R^6 and R^7 are independently selected fr m th group consisting of hydrogen, (C_2-C_6)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6)alkoxy, (C_1-C_6)alkylthio, NR^3R^4 and SR^5, where R^3 and R^4 are as defined above, and R^5 is hydrogen or (C_1-C_6)alkyl, hydroxy-, alkoxy-, or alkylthio- substituted (C_1-C_6)alkyl, or R^6 and R^7 taken together complete an alicyclic or heterocyclic system;

D is CR^6R^7, $CH_2CR^6R^7$ or CHR^6CHR^7, where R^6 and R^7 are as defined above;

E is COOH or an activated or protected derivative thereof; and

F is $NPgR^3$ where R^3 is as defined above and Pg is an amino protecting group;

C) removing said amino protecting group from said coupled first amino acid to generate a free amino group; and

D) reacting said free amino group with a second amin acid having formula (IV) to form a peptide chain.

11. The process of claim 10 further comprising the steps of:

E) removing said amino protecting group from said second amino acid to generate a terminal free amino group n said peptide chain; and

F) reacting said free amino group on said peptide chain with a further amino acid having formula (IV) to lengthen said peptide chain.

12. The process of claim 11 wherein steps E and F are performed a plurality of times.

13. The process of claim 11 further comprising removing at l ast one protecting group remaining on the amino acid moieties of the peptide chain.

14. The pr cess of claim 10 further c mprising cleaving said anch ring linkage without substantially degrading said peptide chain.

15. The process of claim 10 wherein the polymer substrate contains polystyrene, polyacrylamide, silica, a composite material, cotton, or a derivative thereof.

16. The process of claim 10 wherein the chemical group capable of forming said anchoring linkage is chloro-, bromo- and iodo-substituted alkyl, amino-substituted alkyl, amino and aryl-substituted alkyl, amino- and alkylaryl-substituted alkyl, hydroxy-substituted alkyl, or a derivative thereof having a spacer group that can be cleaved substantially without degradation of said polypeptide.

17. The process of claim 16 wherein chloro-substituted alkyl is chloromethyl, amino-substituted alkyl is aminomethyl, amino- and alkyl-substituted aryl is α-aminobenzyl, amino- and alkylaryl-substituted alkyl is selected from the group consisting of α-amino-3- and α-amino-4-methylbenzyl, and hydroxy-substituted alkyl is hydroxymethyl.

18. The process of claim 16 wherein:

the chemical group is derived from an amino-containing moiety selected from amino-substituted alkyl, amino- and aryl substituted alkyl, and amino- and alkylaryl-substituted alkyl; and

the chemical group includes a spacer group derived from the group consisting of 4-(haloalkyl)aryl-lower alkanoic acids, Boc-aminoacyl-4-(oxymethyl)aryl-lower alkanoic acids, N-Boc-p-acylbenzhydrylamines, N-Boc-4′-(lower alkyl)-p-acylbenzhydrylamines, N-Boc-4′-(lower alkoxy)-p-acylbenzhydrylamines, and 4-hydr xymethylphenoxy-lower alkanoic acids.

19. A process for sequence-sp cific recognition of a d ubl -stranded polynucleotide, comprising contacting said polynucleotide with a compound that is different from natural RNA and that binds to one strand of the polynucleotide, thereby displacing the other strand.

20. The process of claim 19 wherein said compound is an oligomer comprising a homogenous or heterogenous backbone to which are linked naturally occurring nucleobases, non-naturally occurring nucleobases or other ligands that individually bind by hydrogen to at least one natural nucleobase in said bound polynucleotide strand.

21. The process of claim 20 wherein said compound is the compound of claim 1.

22. The process of claim 20 wherein said compound is the compound of claim 4.

23. A process for modulating the expression of a gene in an organism, comprising administering to said organism a compound according to claim 1 that specifically binds to DNA or RNA deriving from said gene.

24. The process of claim 23 wherein said compound is the compound of claim 1.

25. The process of claim 23 wherein said compound is the compound of claim 4.

26. The process of claim 23 wherein said modulation includes inhibiting transcription of said gene.

27. Th process of claim 23 wherein said modulation includes inhibiting replication of said gene.

28. A pr cess for treating conditions associated with undesired protein production in an organism, c mprising contacting said organism with an effective amount of a compound according to claim 1 that specifically binds with DNA or RNA deriving from a gene controlling said protein production.

29. The process of claim 28 wherein said compound is the compound of claim 1.

30. The process of claim 28 wherein said compound is the compound of claim 4.

31. A process for inducing degradation of DNA or RNA in cells of an organism, comprising administering to said organism a compound according to claim 1 that specifically binds to said DNA or RNA.

32. A process for killing cells or virus, comprising contacting said cells or virus with a compound according to claim 1 that specifically binds to a portion of the genome of said cells or virus.

33. A pharmaceutical composition comprising a compound according to claim 1 and at least one pharmaceutically effective carrier, binder, thickener, diluent, buffer, preservative, or surface active agent.

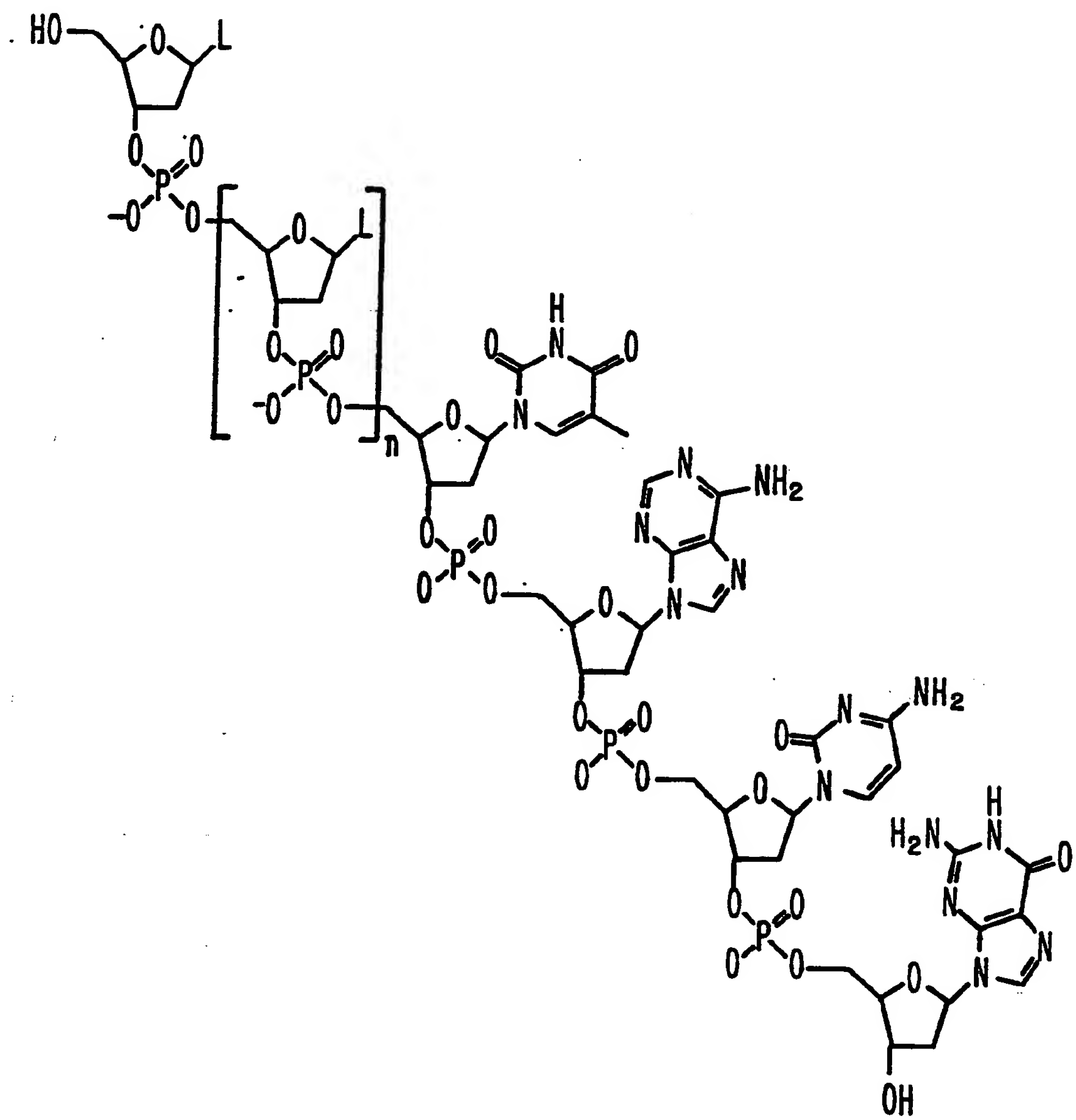


Fig. 1(A)

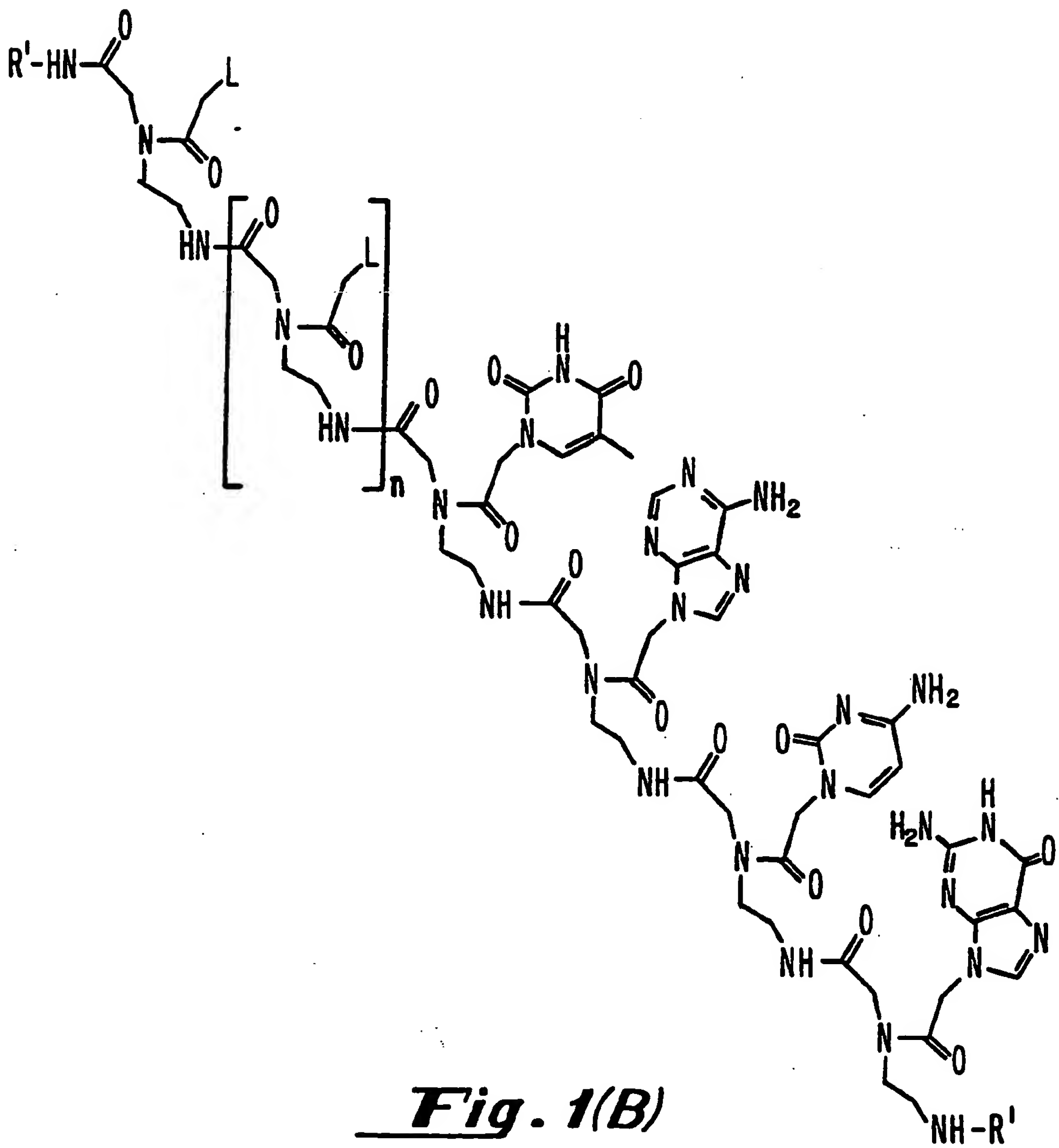

R'-HN
O
L
N
O
HN
O
L
N
O
HN
n
O
H
O
N
O
N
N
O
N
NH2
N
NH
O
N
N
N
O
N
NH2
O
N
NH
O
N
H
H2N
N
O
N
O
N
NH
O
N
N
N
O
NH-R'

Fig. 1(B)

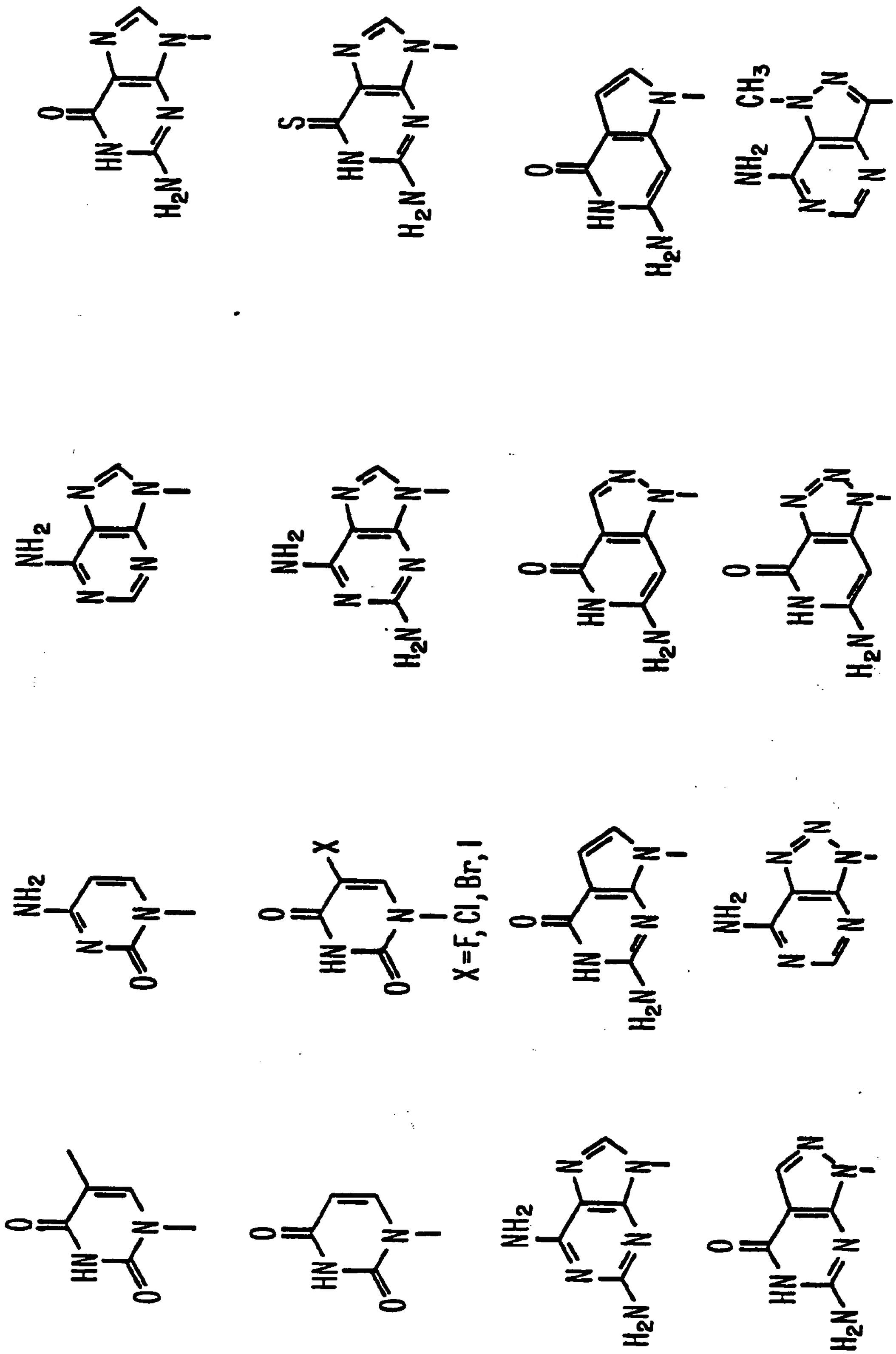
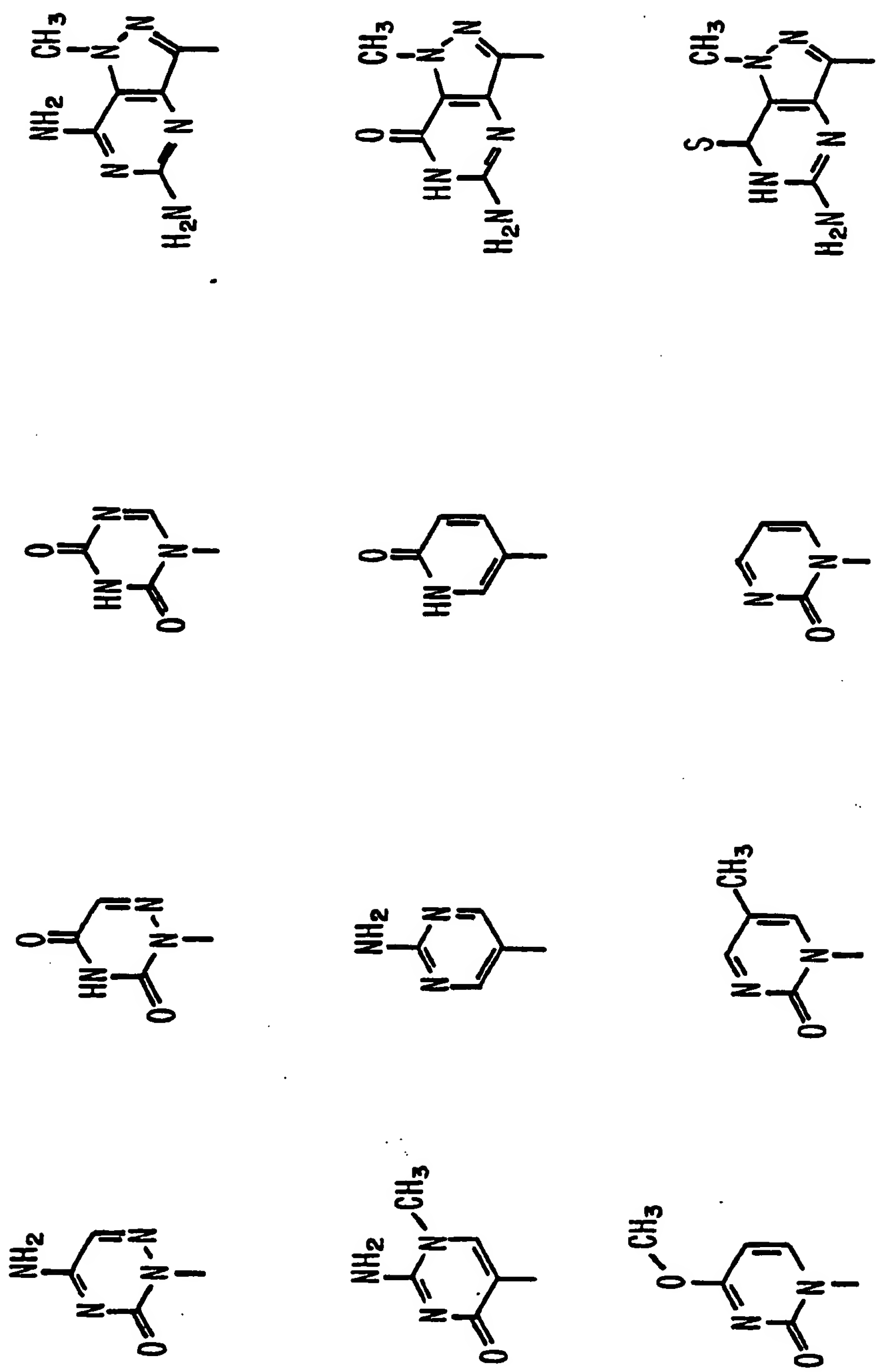


Fig. 2(A)


CH3
NH2
H2N
N
CH3
O
HN
H2N
N
CH3
S
HN
H2N
N
O
HN
O
N
O
HN
N
O
O
HN
O
N
NH2
N
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NH2
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O
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CH3
N
O

Fig. 2(B)

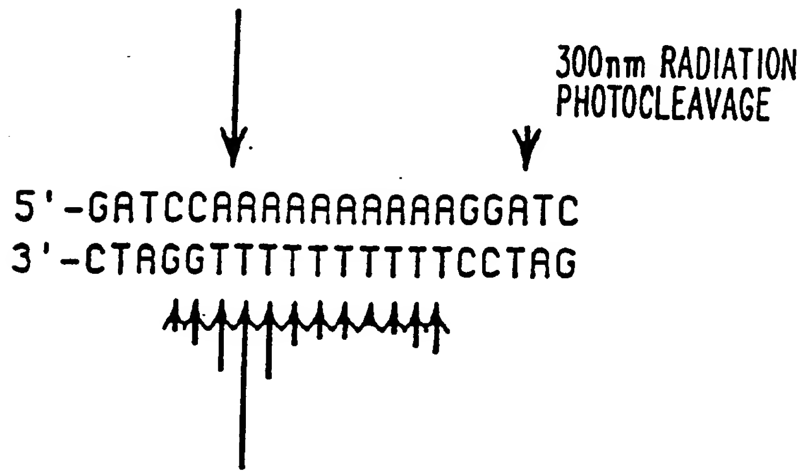


Fig. 3(a)

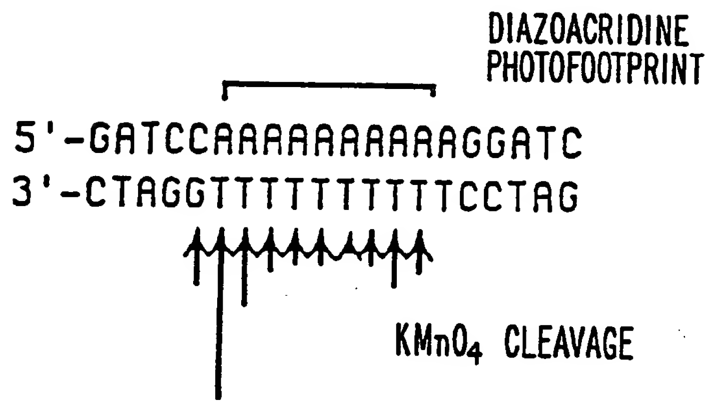


Fig. 3(b)

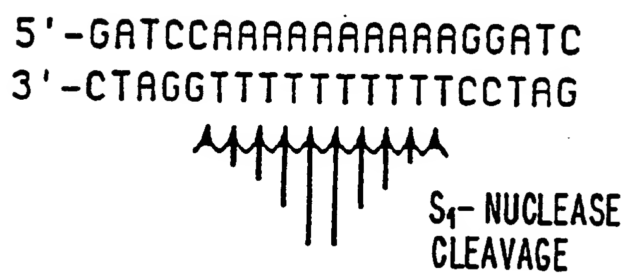


Fig. 3(c)

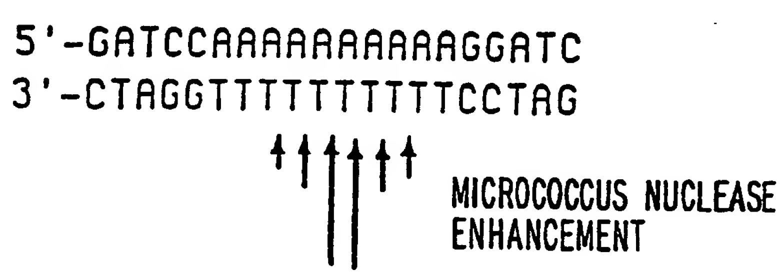


Fig. 3(d)

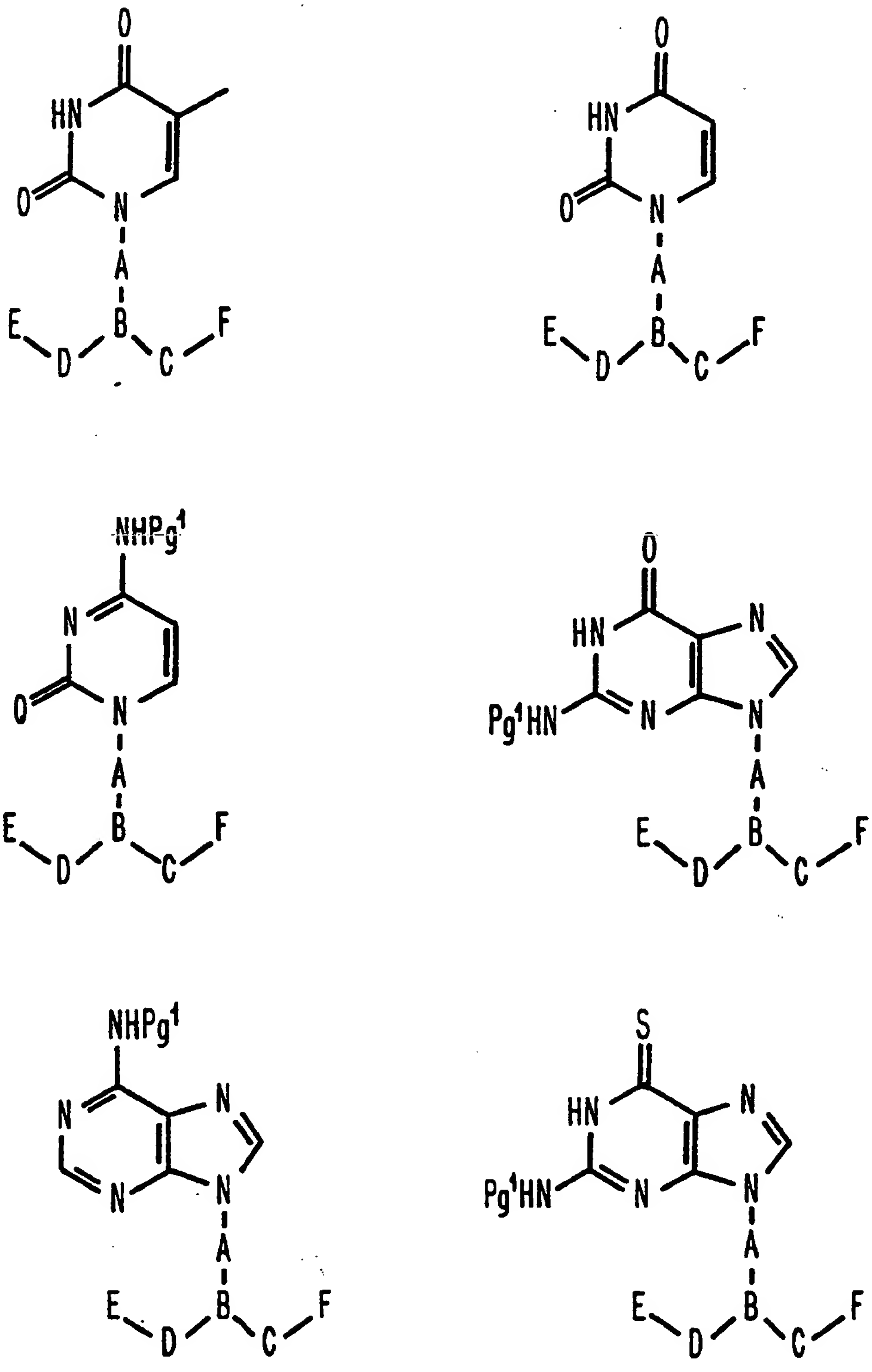
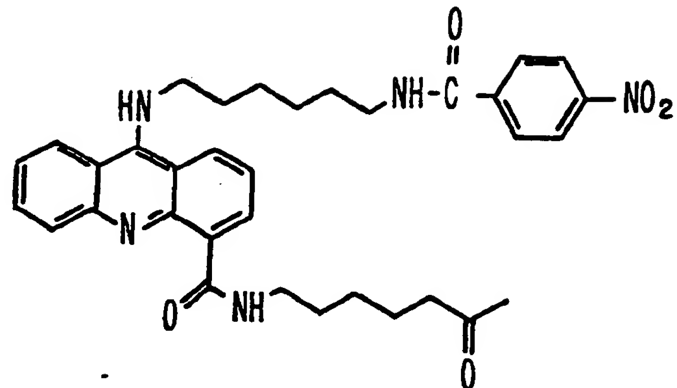


Fig. 4



Acr[1]

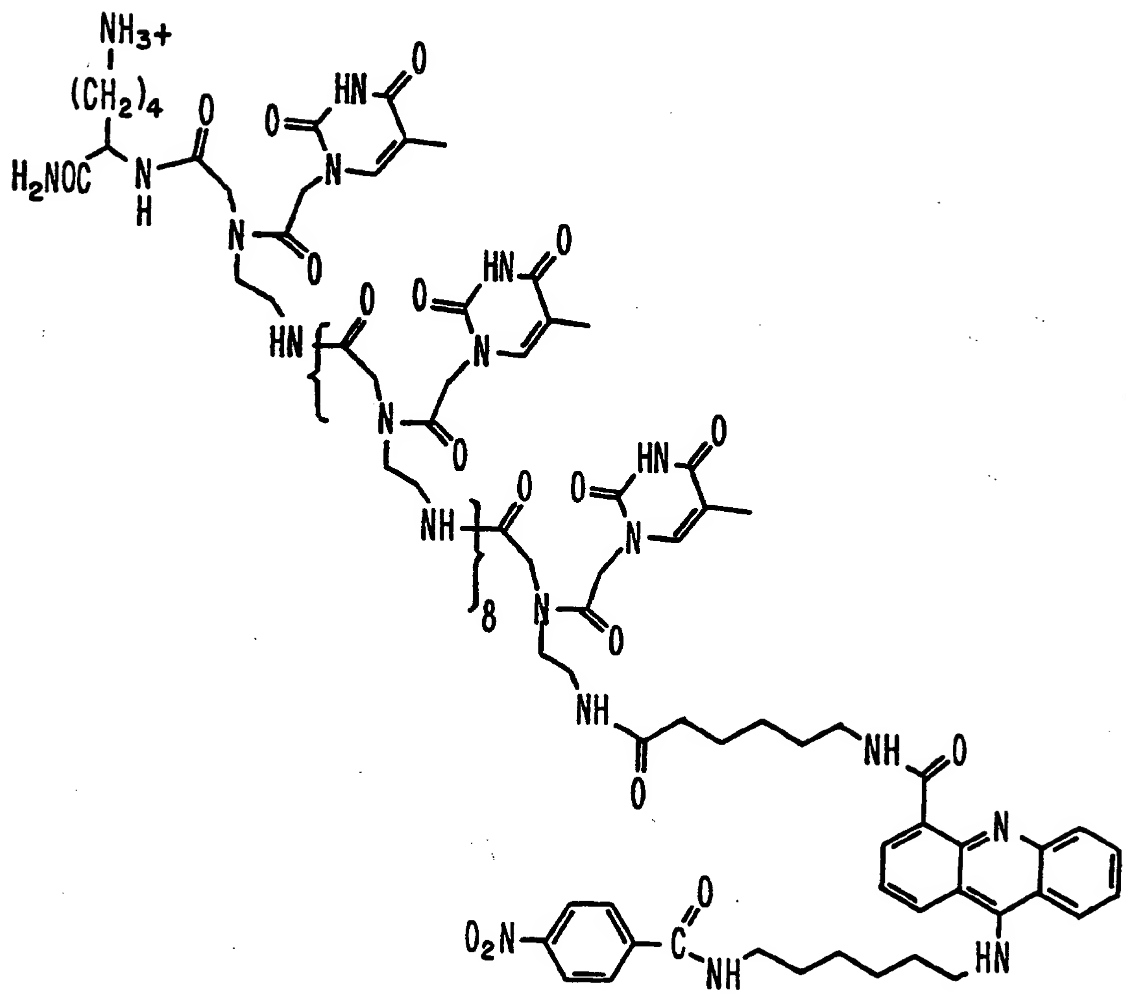


Fig. 5

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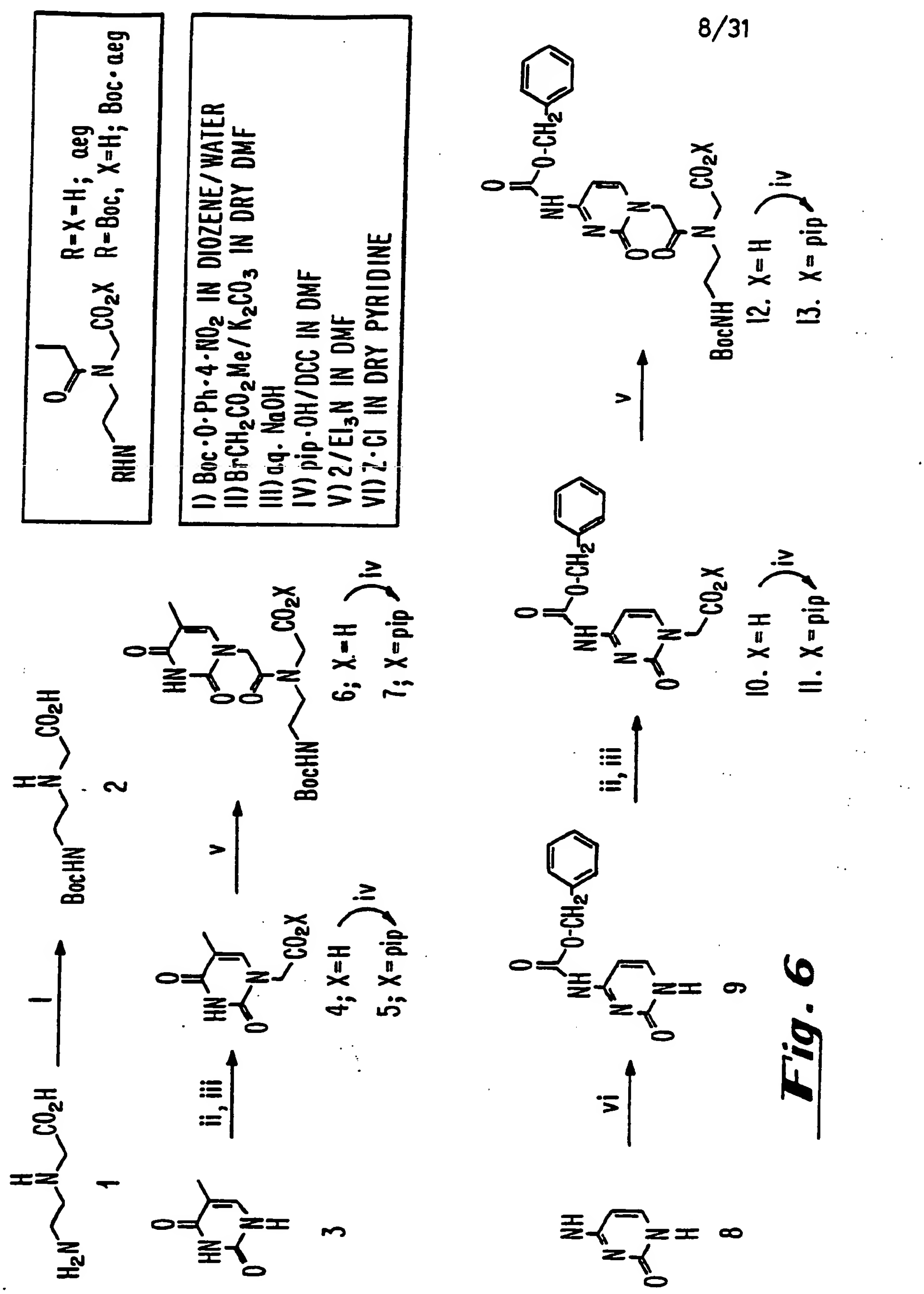

RHN
CO2X
R=X=H; aeg
R=Boc, X=H; Boc·aeg
I) Boc·O·Ph·4·NO2 IN DIOZENE/WATER
II) BrCH2CO2Me/K2CO3 IN DRY DMF
III) aq. NaOH
IV) pip·OH/DCC IN DMF
V) 2/Et3N IN DMF
VI) Z·Cl IN DRY PYRIDINE
1
2
3
4; X=H
5; X=pip
6; X=H
7; X=pip
8
9
10. X=H
11. X=pip
12. X=H
13. X=pip
i
ii, iii
iv
v
vi

Fig. 6

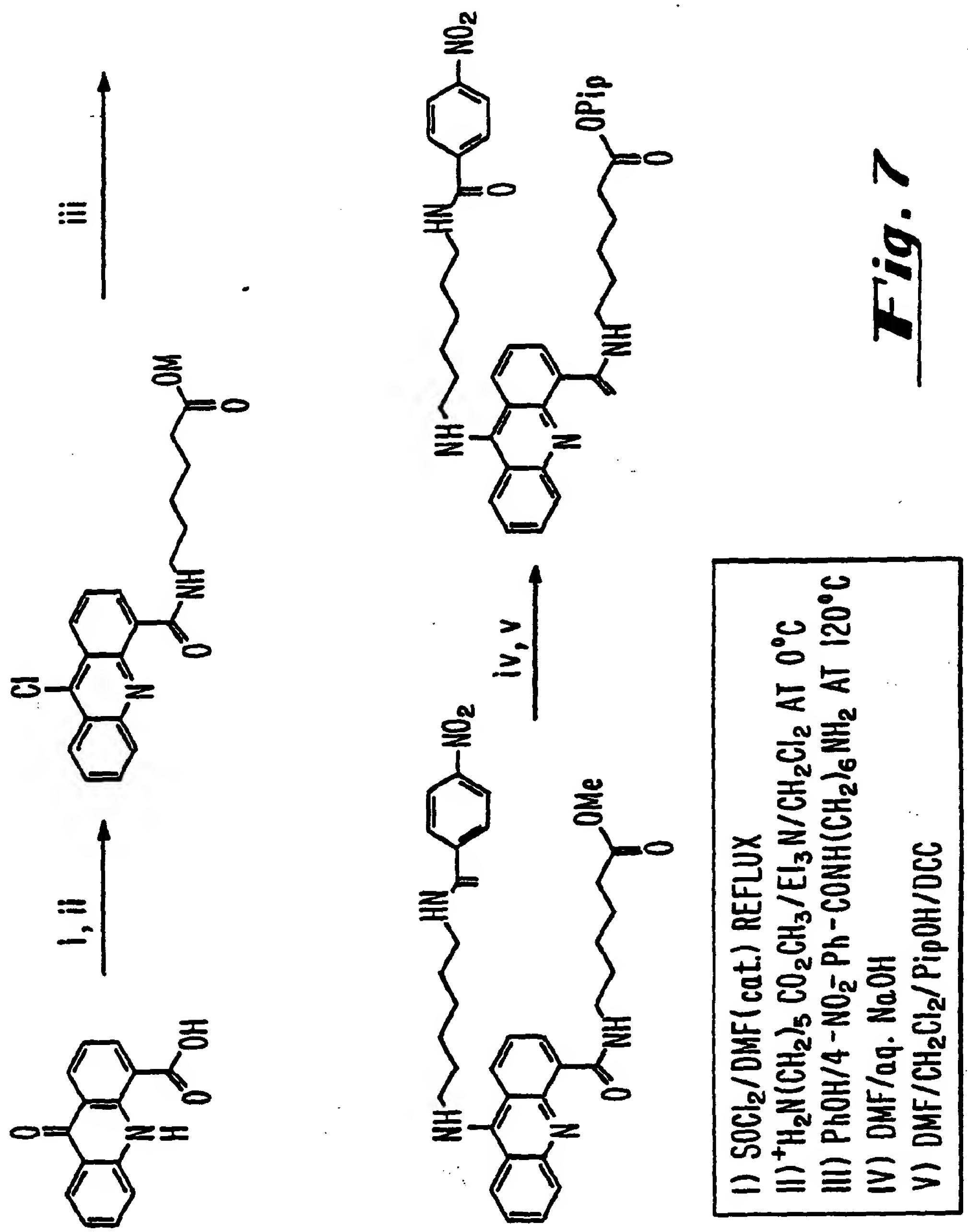
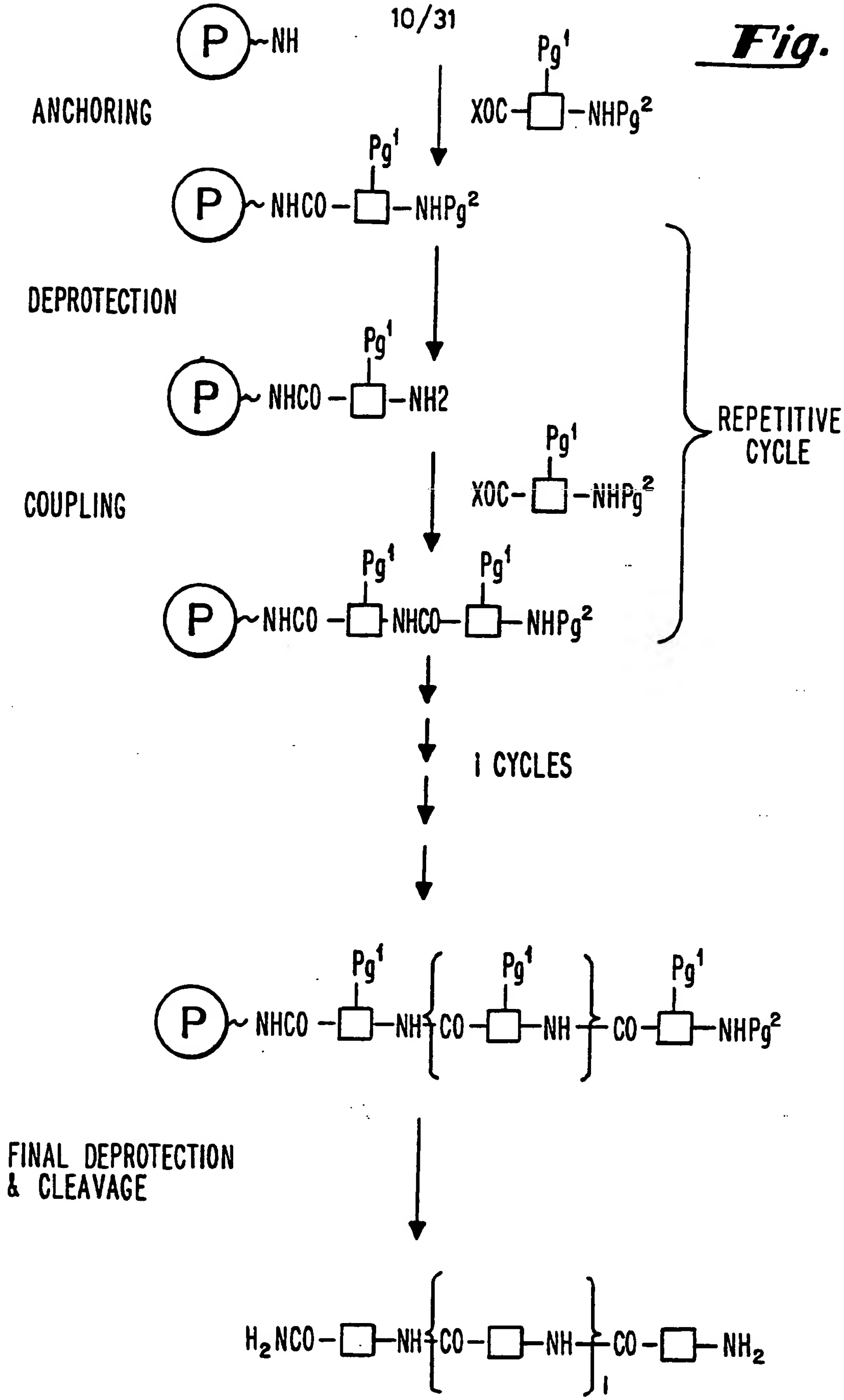

i, ii
iii
iv, v
Cl
N
NH
O
OMe
NO2
HN
OPip
I) SOCl2/DMF(cat.) REFLUX
II) +H2N(CH2)5 CO2CH3/Et3N/CH2Cl2 AT 0°C
III) PhOH/4-NO2-Ph-CONH(CH2)6NH2 AT 120°C
IV) DMF/aq. NaOH
V) DMF/CH2Cl2/PipOH/DCC

Fig. 7

Fig. 8



P
NH
ANCHORING
XOC
Pg1
NHPg2
NHCO
DEPROTECTION
NH2
COUPLING
REPETITIVE CYCLE
i CYCLES
FINAL DEPROTECTION & CLEAVAGE
H2NCO
CO
NH
NH2

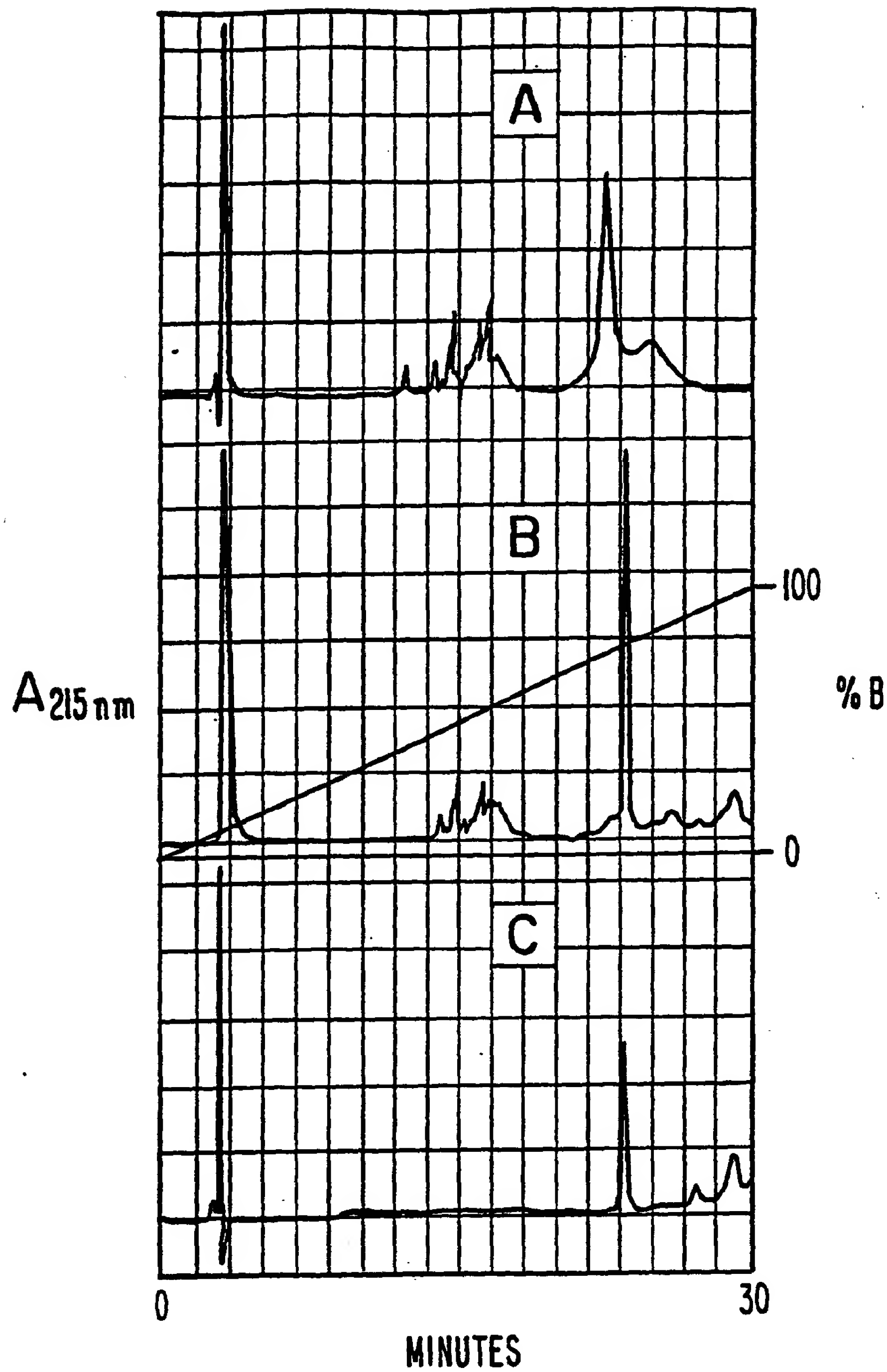


Fig. 9

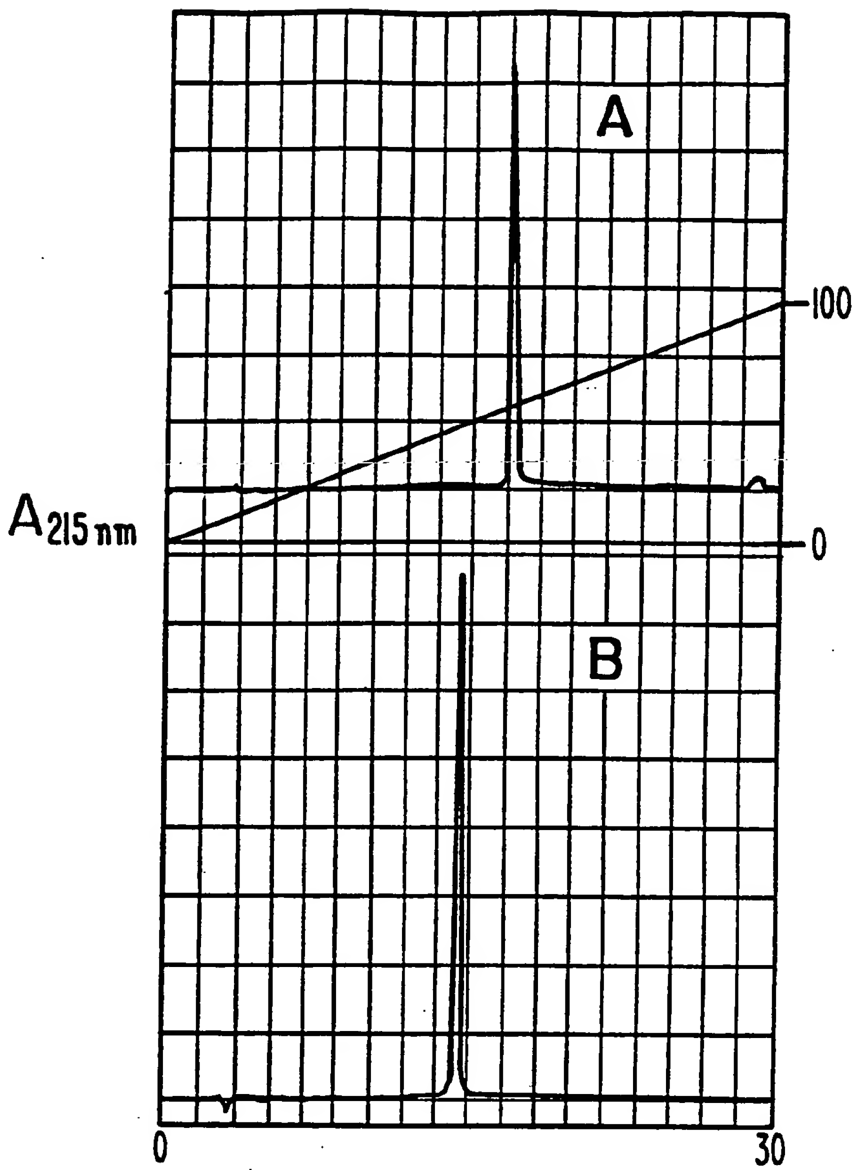


Fig. 10

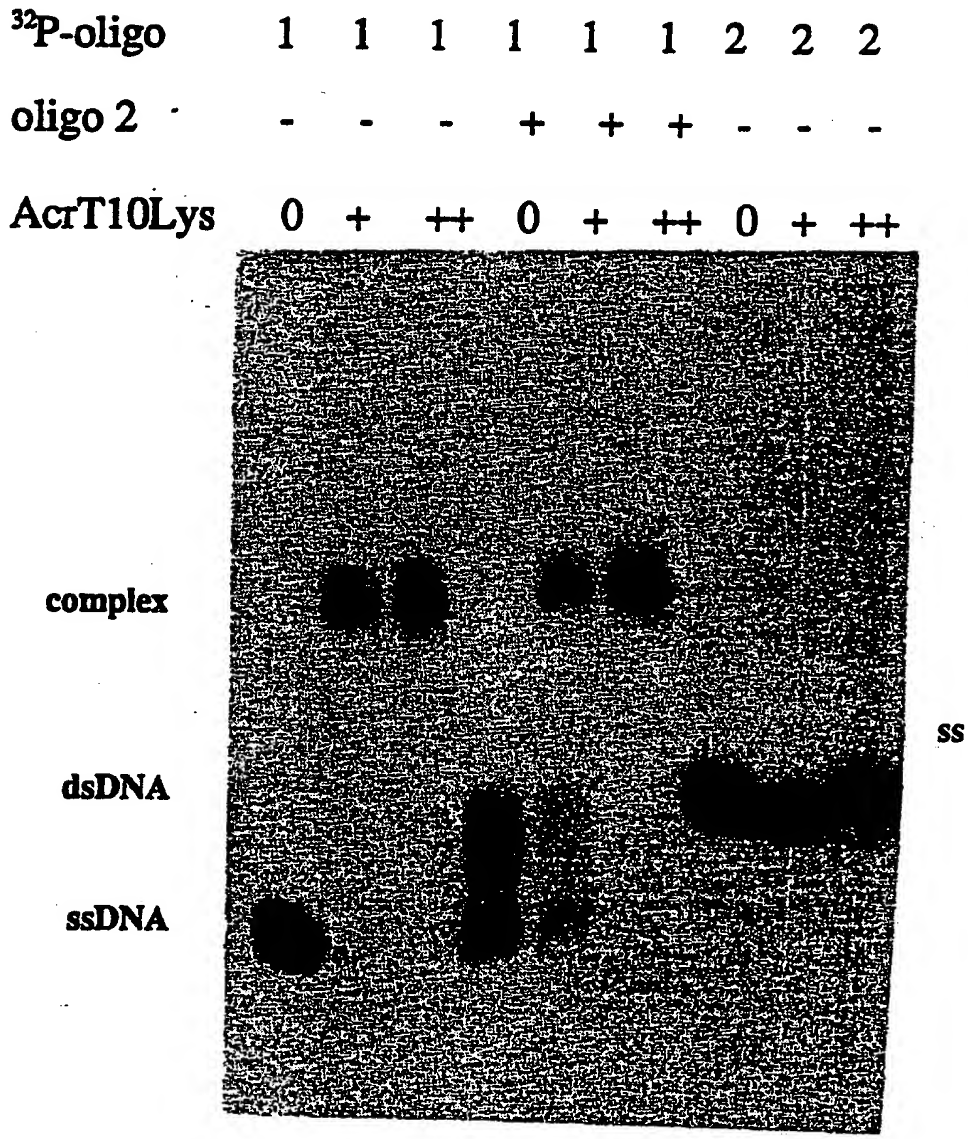

^{32}P-oligo 1 1 1 1 1 1 2 2 2
oligo 2 - - - + + + - - -
AcrT10Lys 0 + ++ 0 + ++ 0 + ++
complex
dsDNA
ssDNA
ss

FIG. 11A

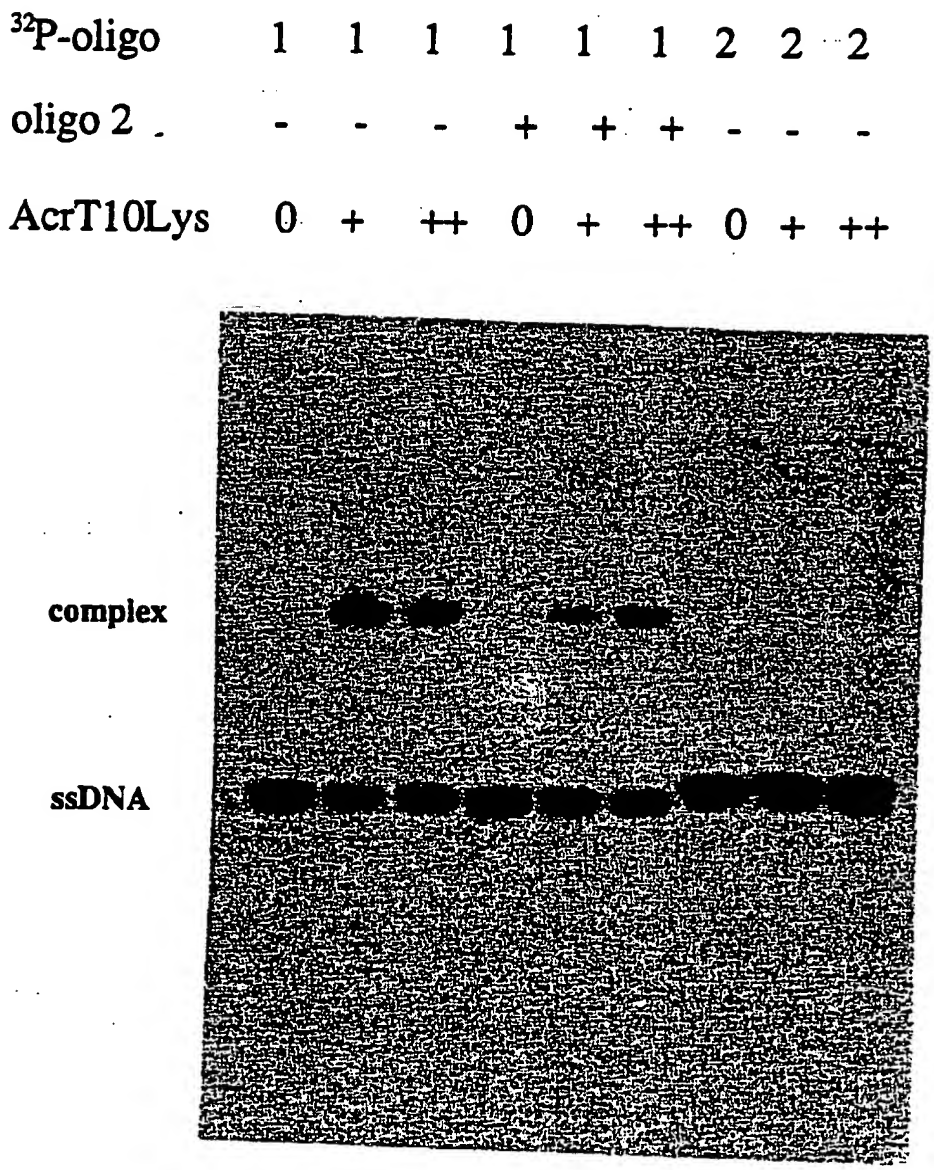


FIG. 11B

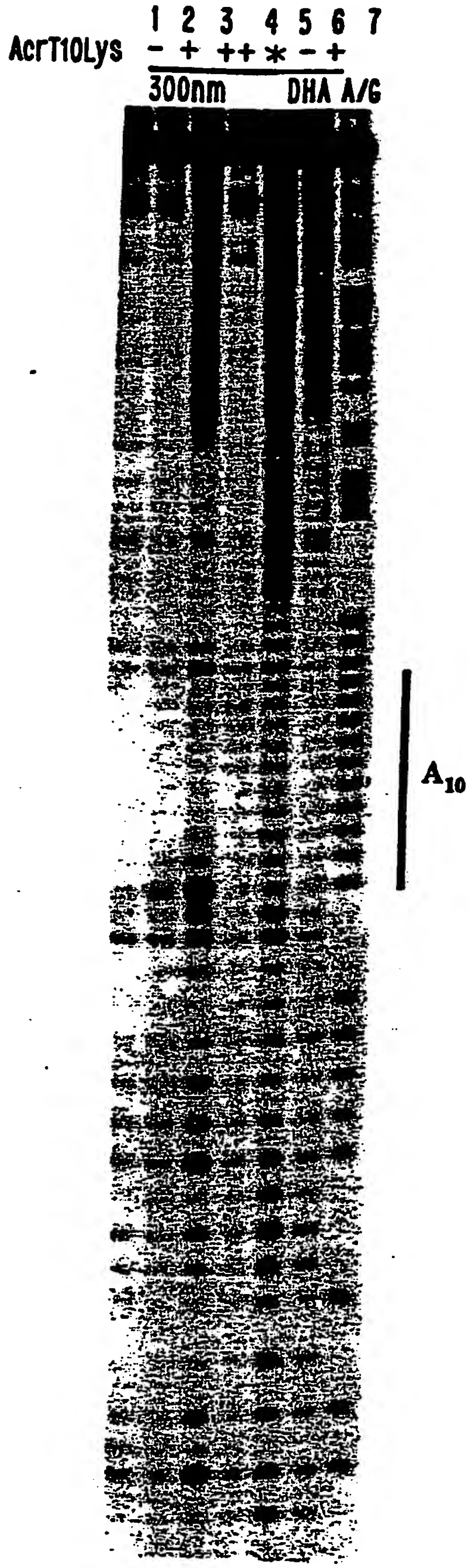


FIG. 12A

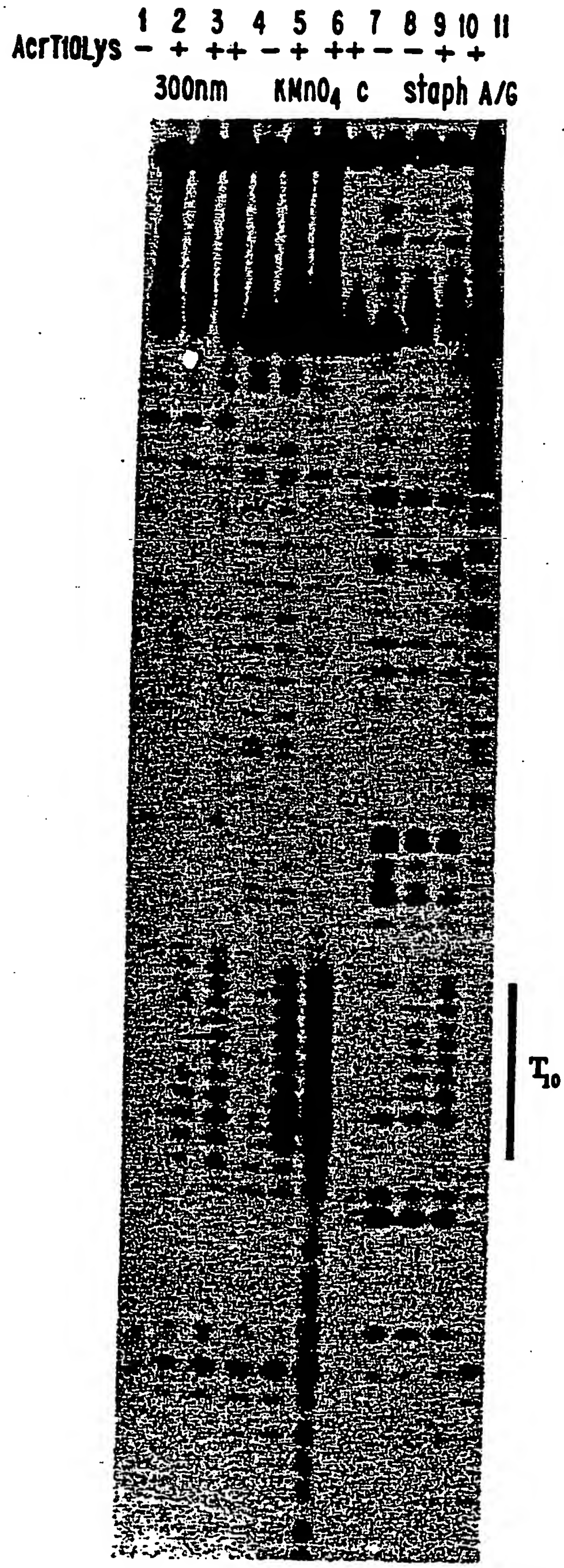


FIG. 12B

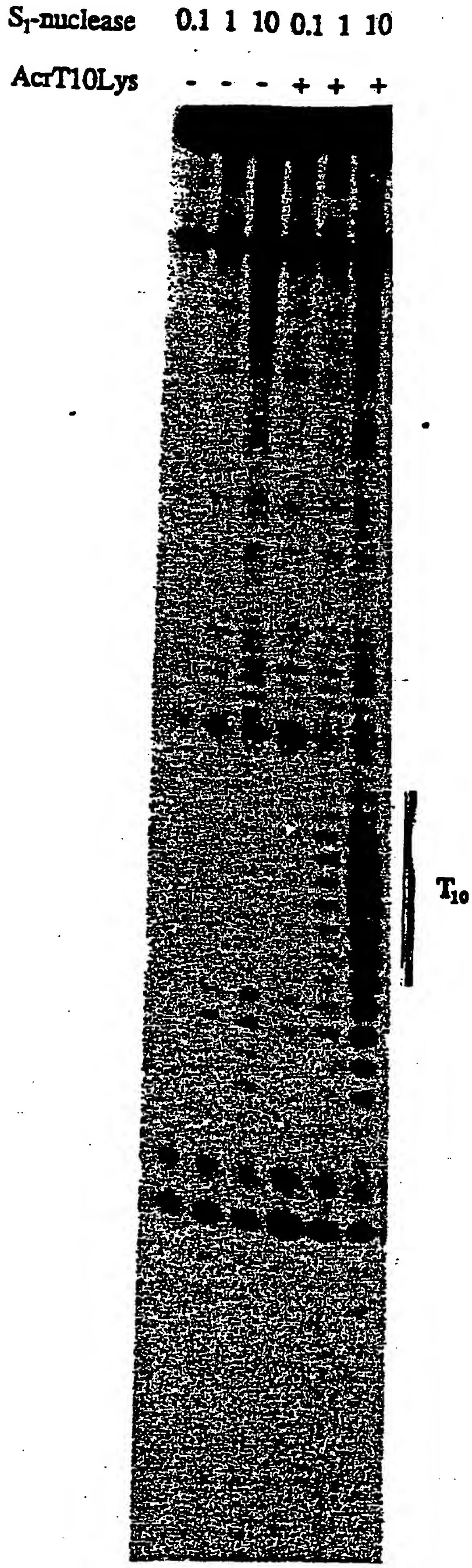
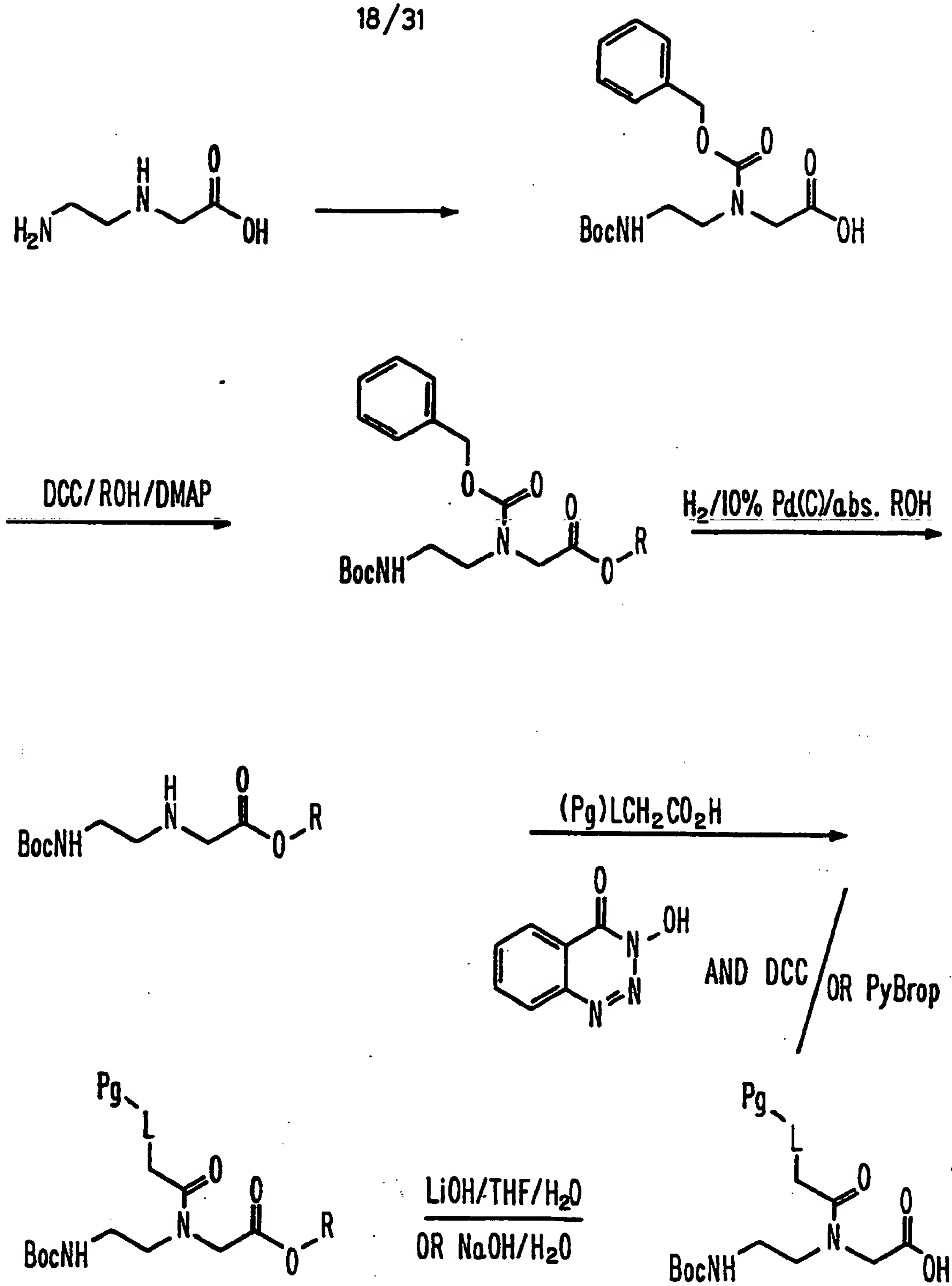


FIG. 12C



H₂N
H
N
O
OH
BocNH
O
O
N
DCC/ROH/DMAP
H₂/10% Pd(C)/abs. ROH
R
(Pg)LCH₂CO₂H
N
OH
N
N
AND DCC
OR PyBrop
Pg
L
LiOH/THF/H₂O
OR NaOH/H₂O

Fig. 13

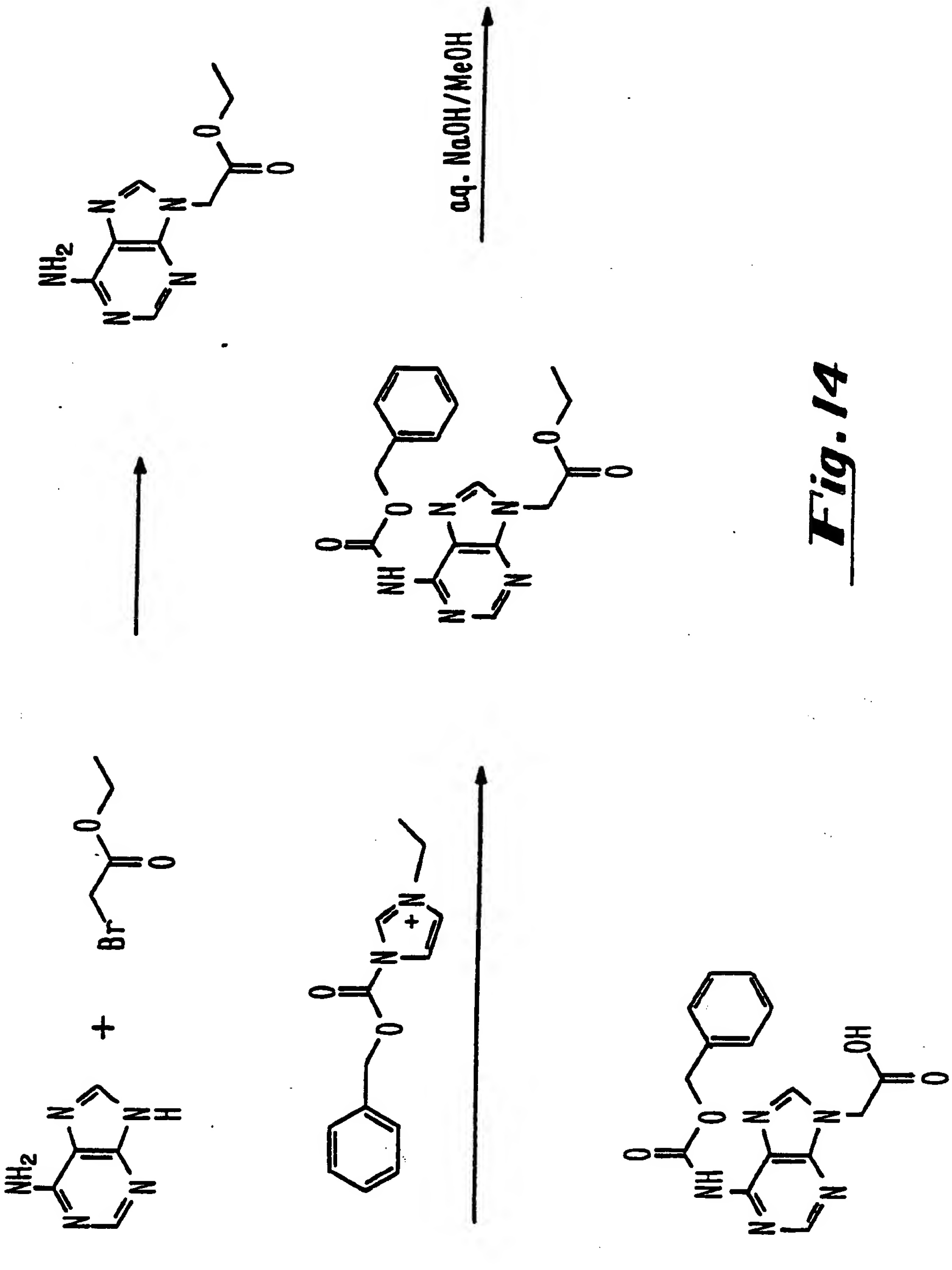


Fig. 14

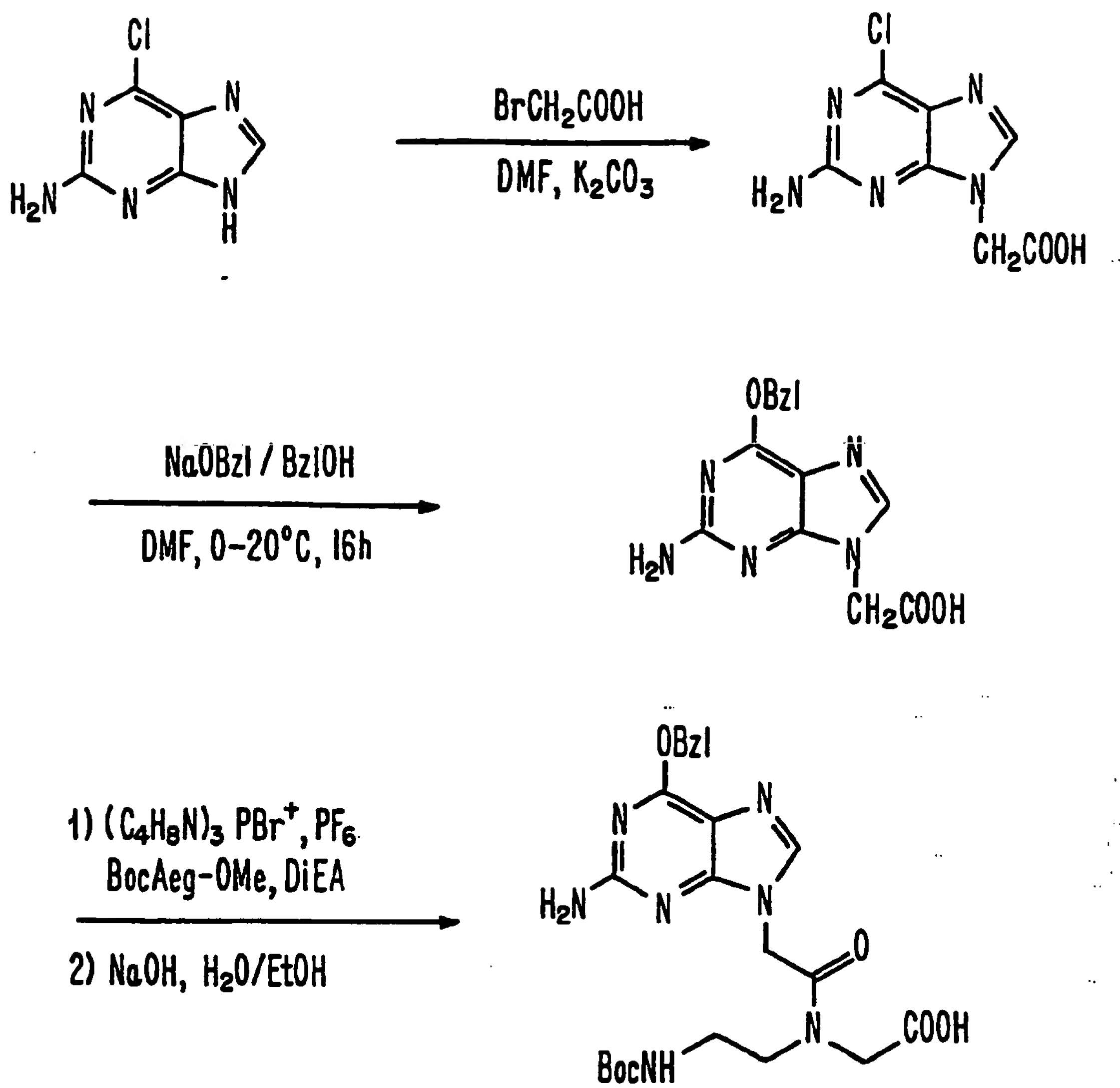

Cl
N
N
H2N
N
N
H
BrCH2COOH
DMF, K2CO3
CH2COOH
NaOBzl / BzlOH
DMF, 0-20°C, 16h
OBzl
1) (C4H8N)3 PBr+, PF6
BocAeg-OMe, DiEA
2) NaOH, H2O/EtOH
O
COOH
BocNH

Fig. 15

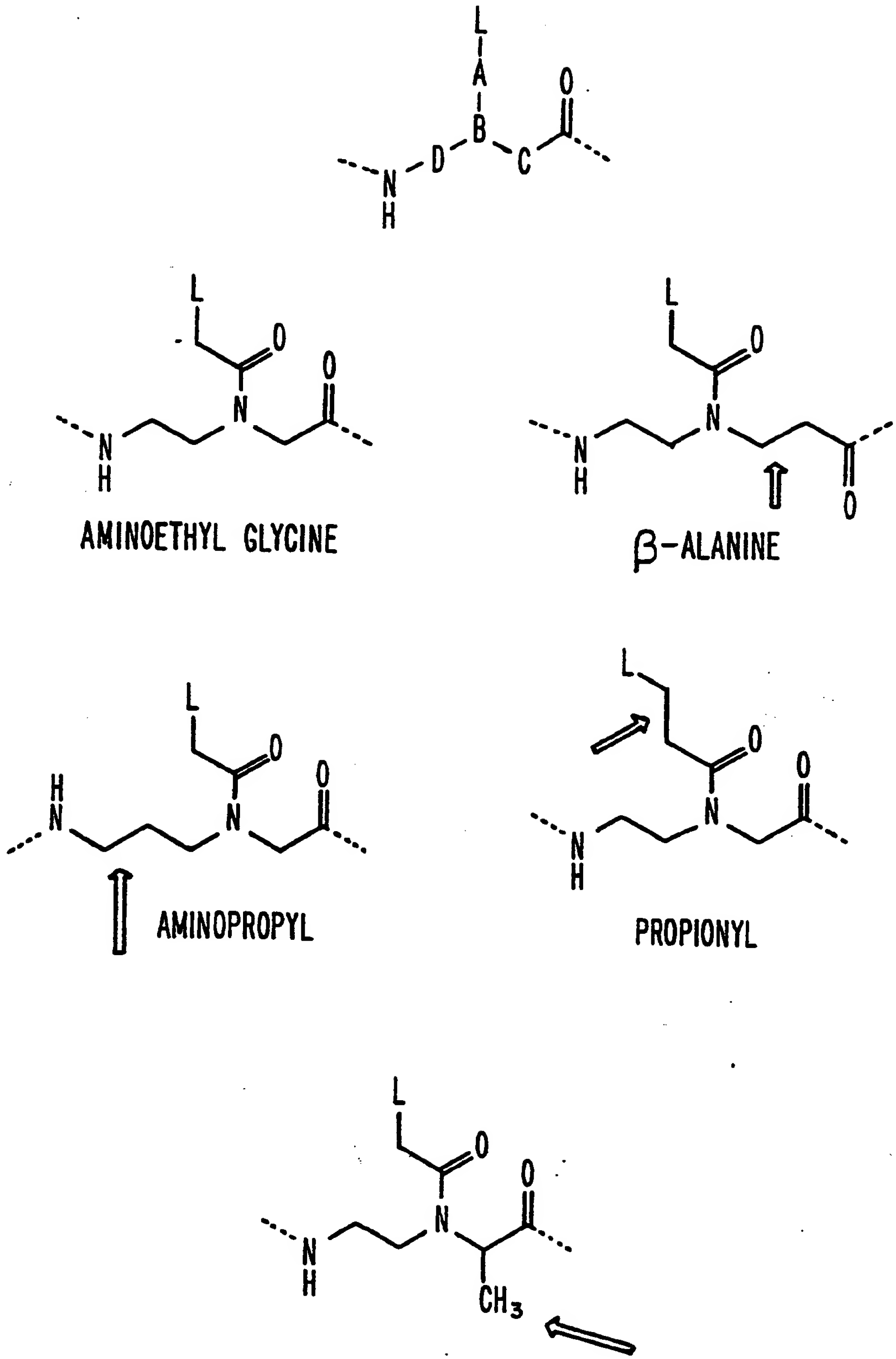


Fig. 16

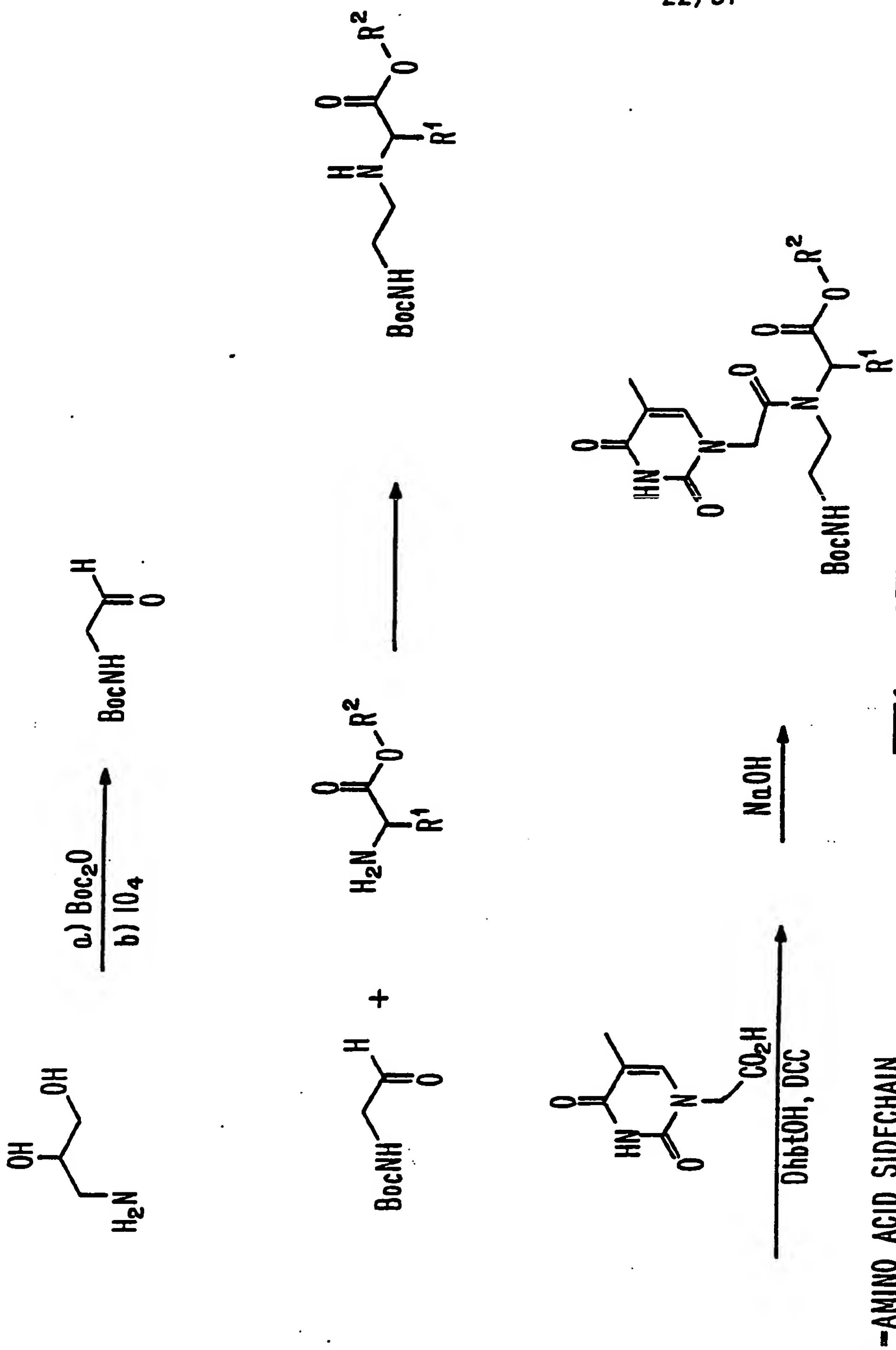


Fig. 17

R^1=AMINO ACID SIDECHAIN
R^2=METHYL, ETHYL ETC.

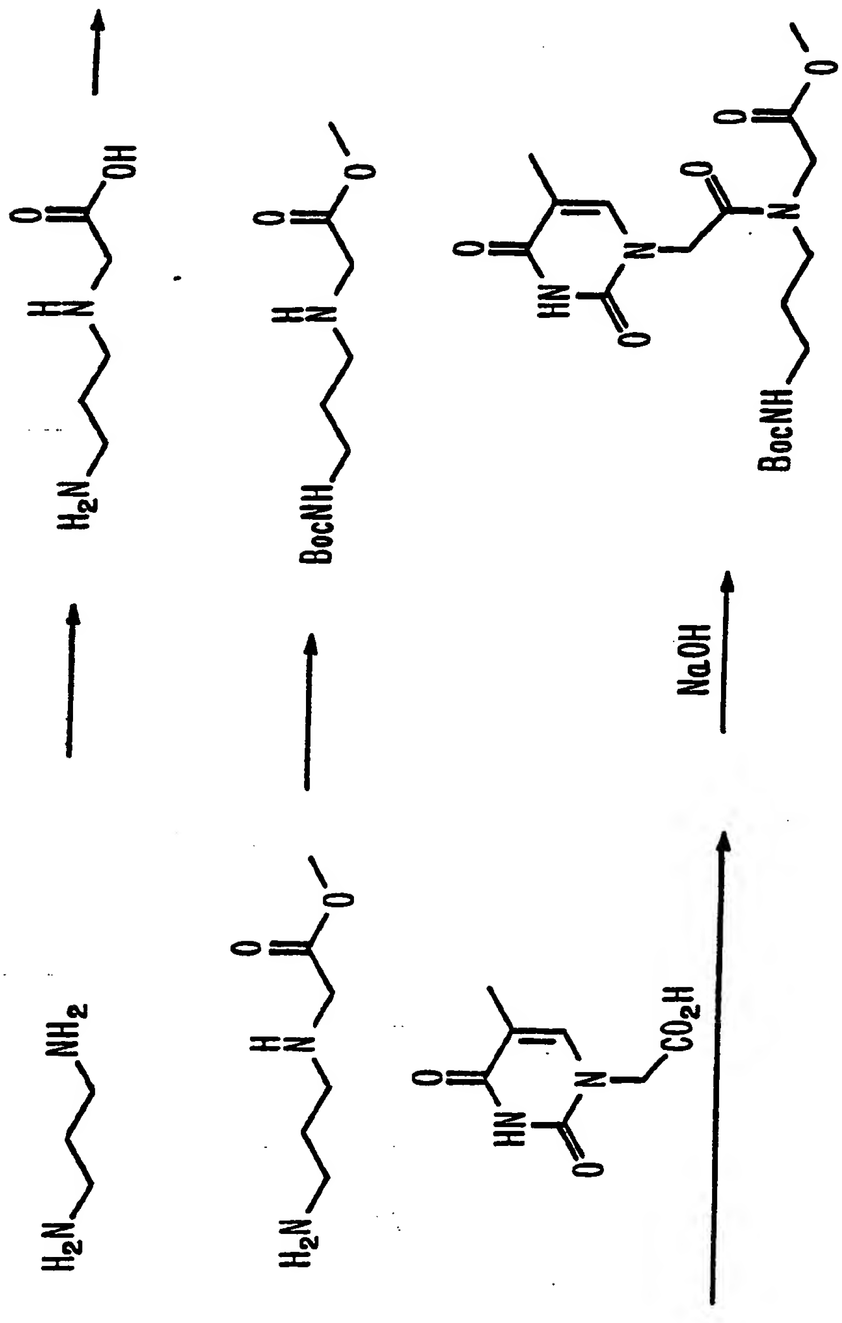


Fig. 18(a)

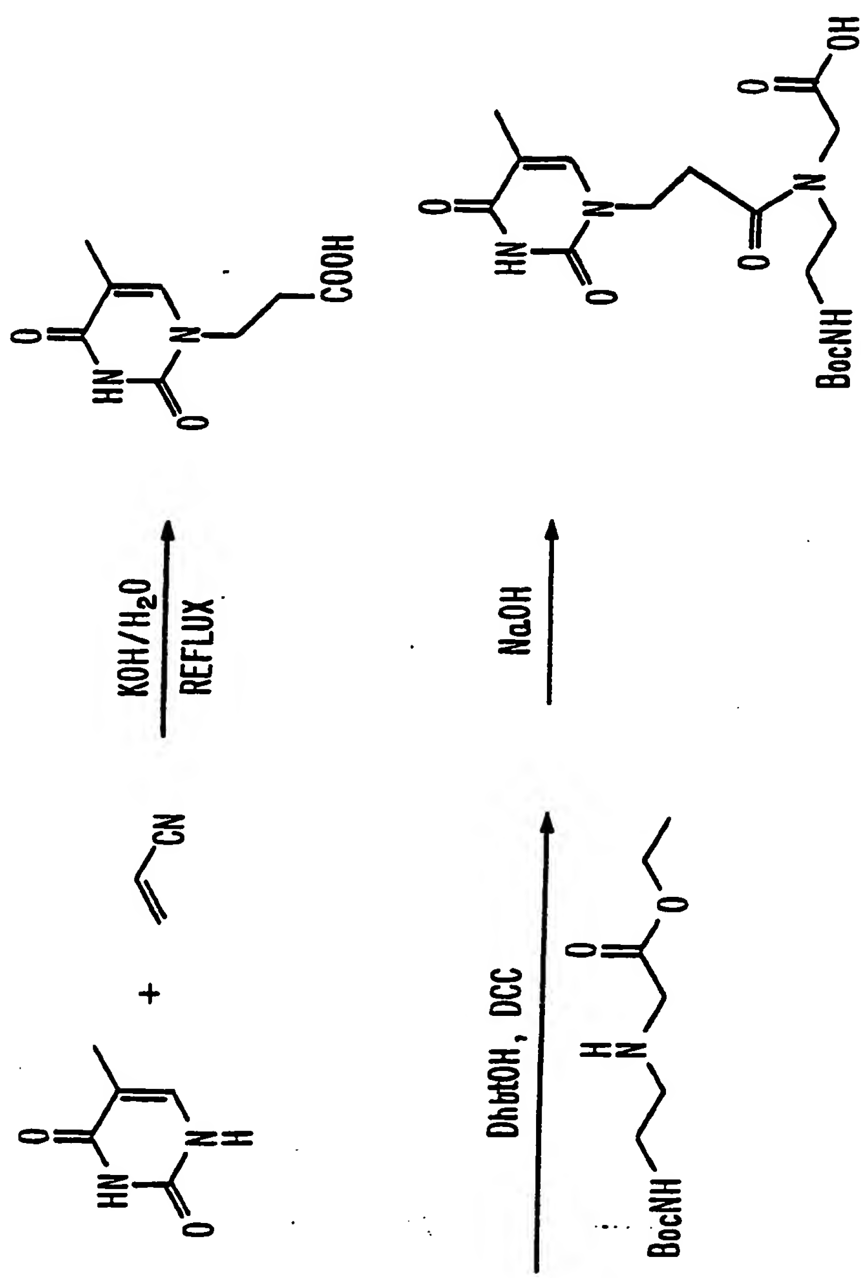


Fig. 18(b)

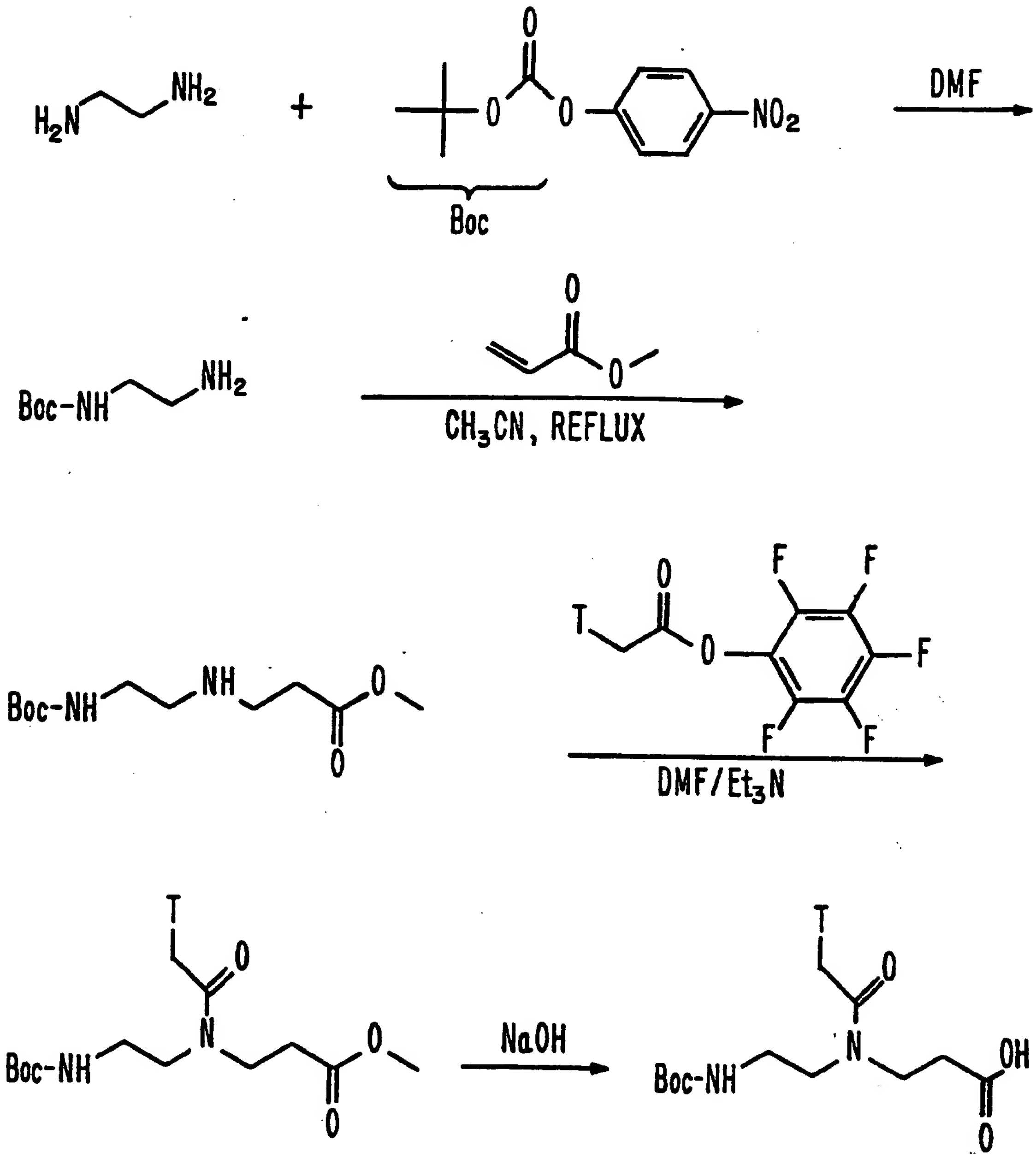


Fig. 19

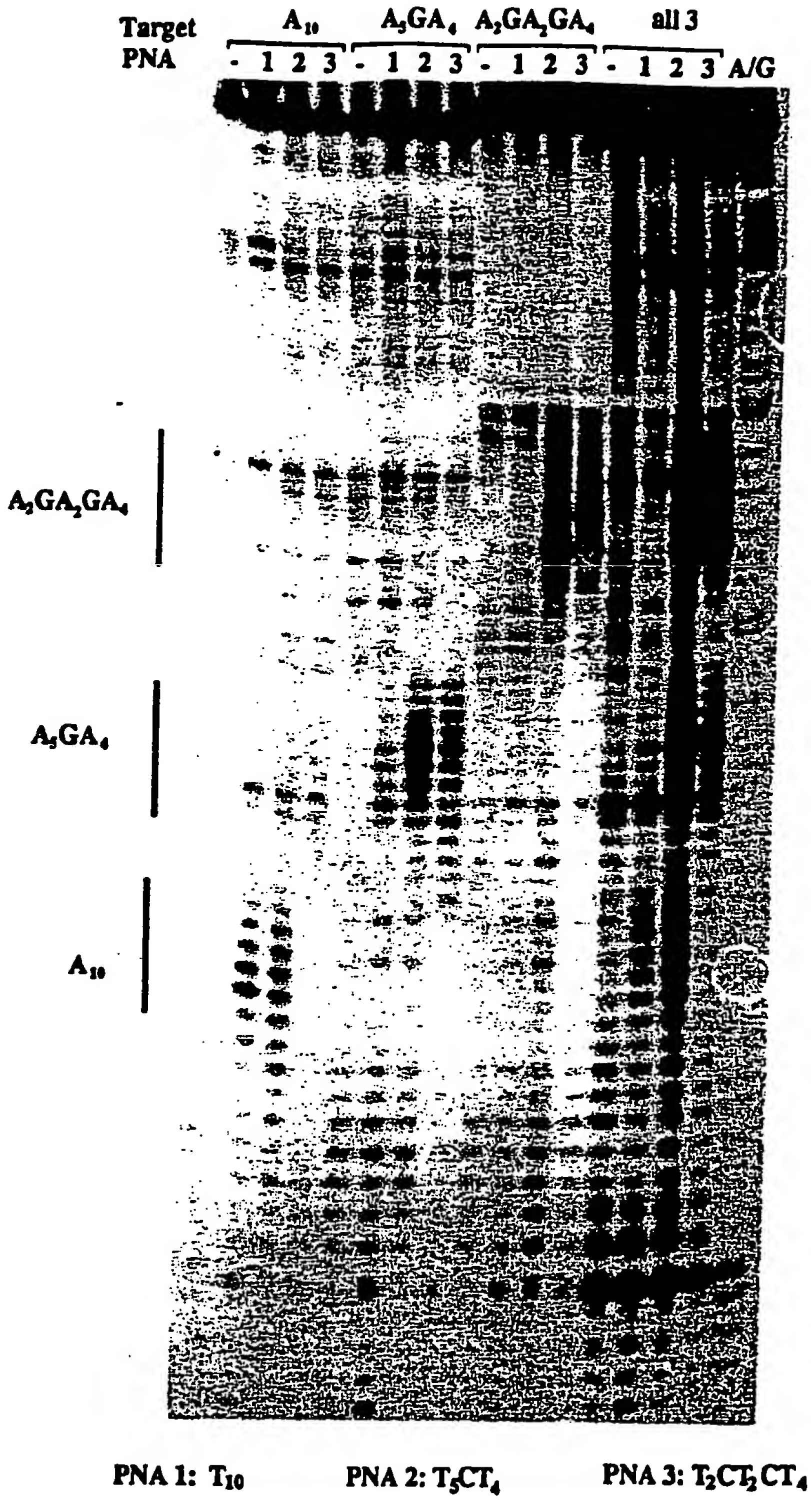

Target
A_{10}
A_5GA_4
$A_2GA_2GA_4$
all 3
PNA
- 1 2 3 - 1 2 3 - 1 2 3 - 1 2 3 A/G
$A_2GA_2GA_4$
A_5GA_4
A_{10}
PNA 1: T_{10}
PNA 2: T_5CT_4
PNA 3: $T_2CT_2CT_4$

FIG. 20

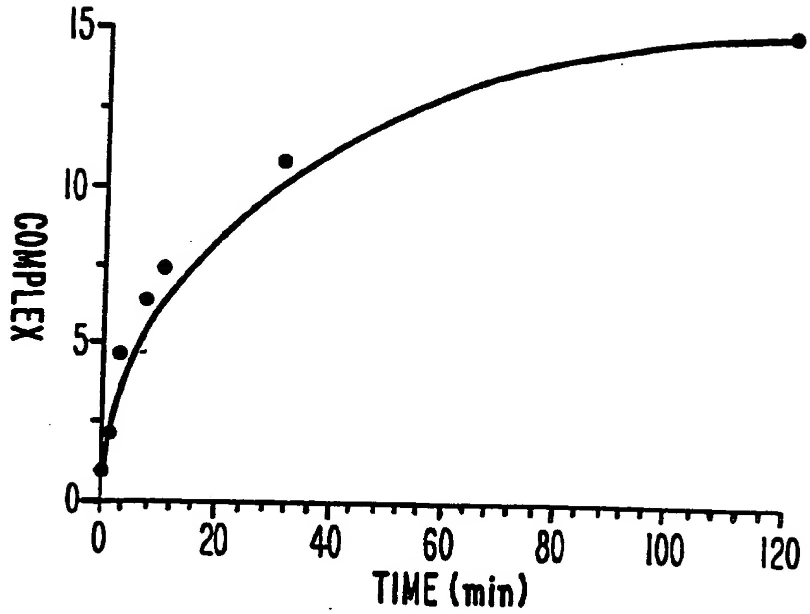

COMPLEX
15
10
5
0
0
20
40
60
80
100
120
TIME (min)

Fig. 21

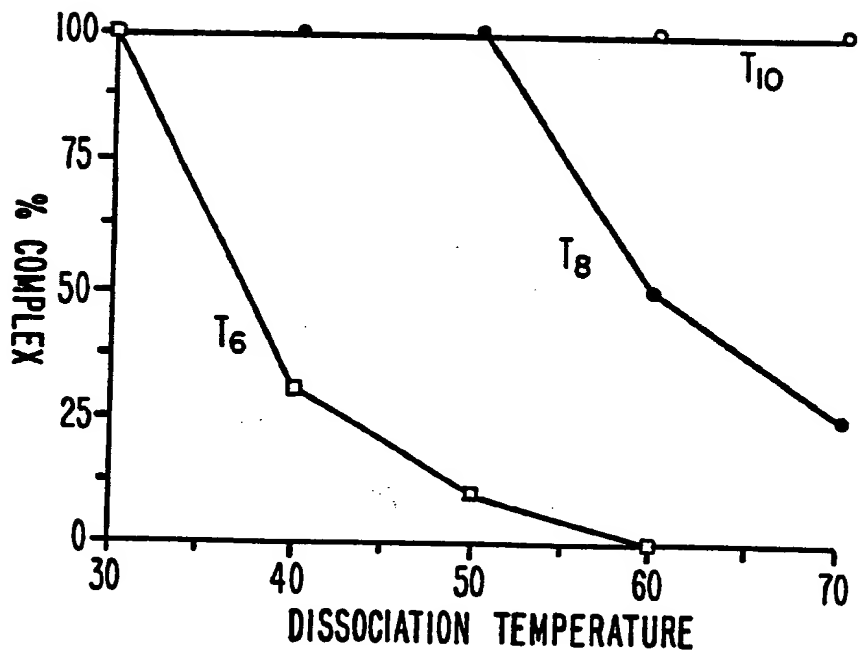
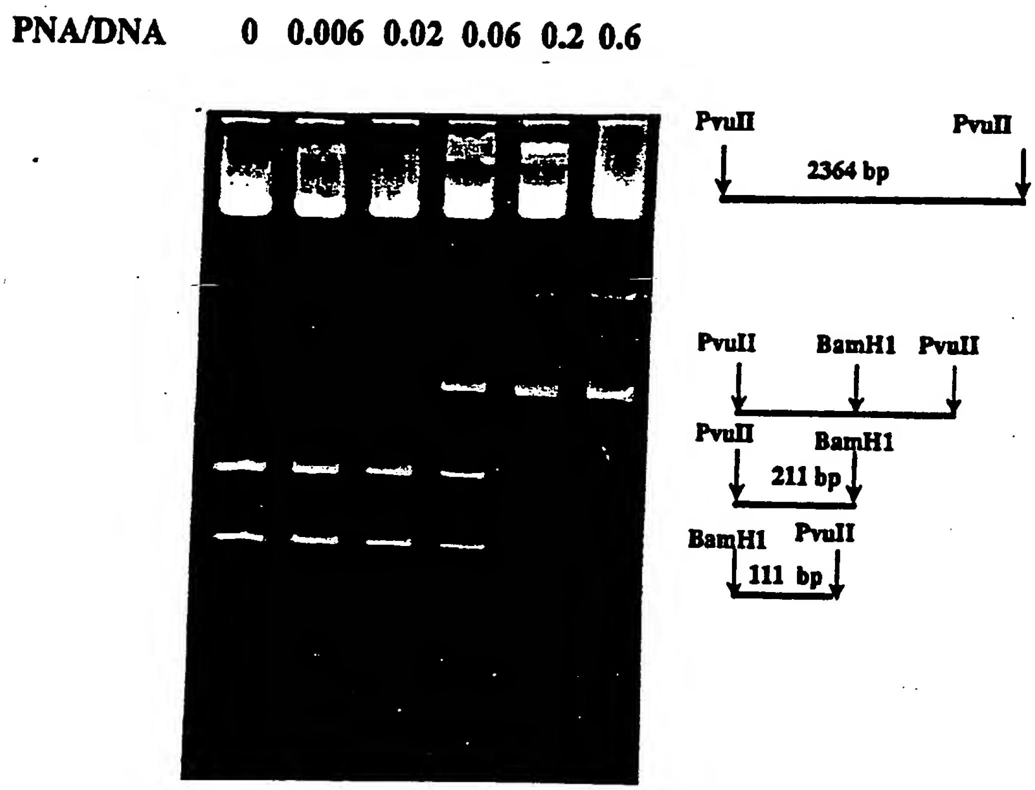

% COMPLEX
100
75
50
25
0
30
40
50
60
70
DISSOCIATION TEMPERATURE
T_{10}
T_8
T_6

Fig. 22



PNA Target

```
5'-------GGATCCAAAAAAAAAAGGATCC-------
3'-------CCTAGGTTTTTTTTTTCCTAGG-------
```

BamH1 BamH1

FIG. 23

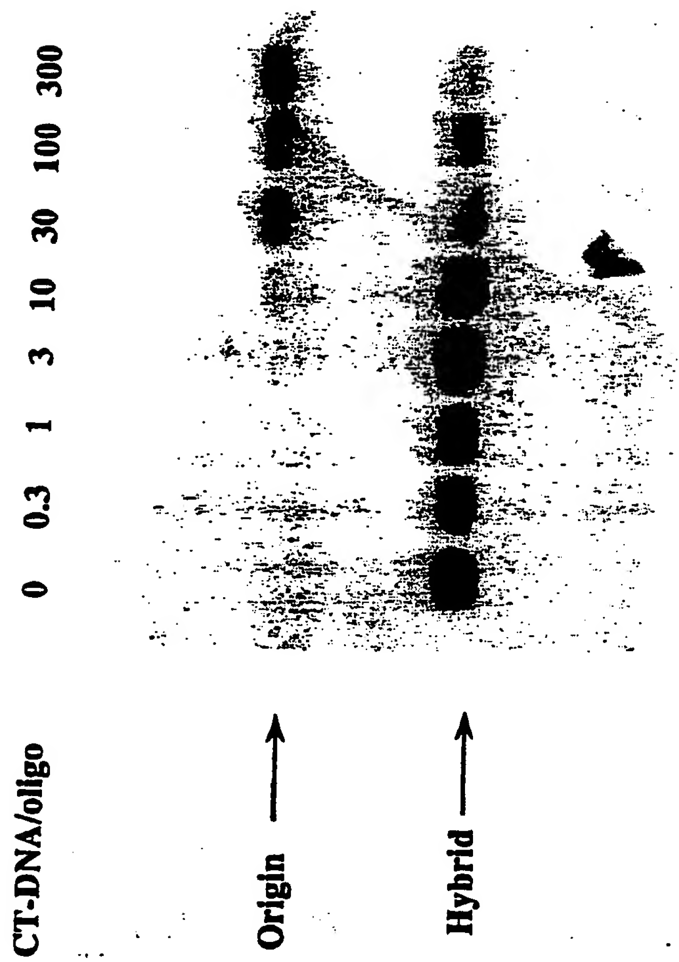


FIG. 24

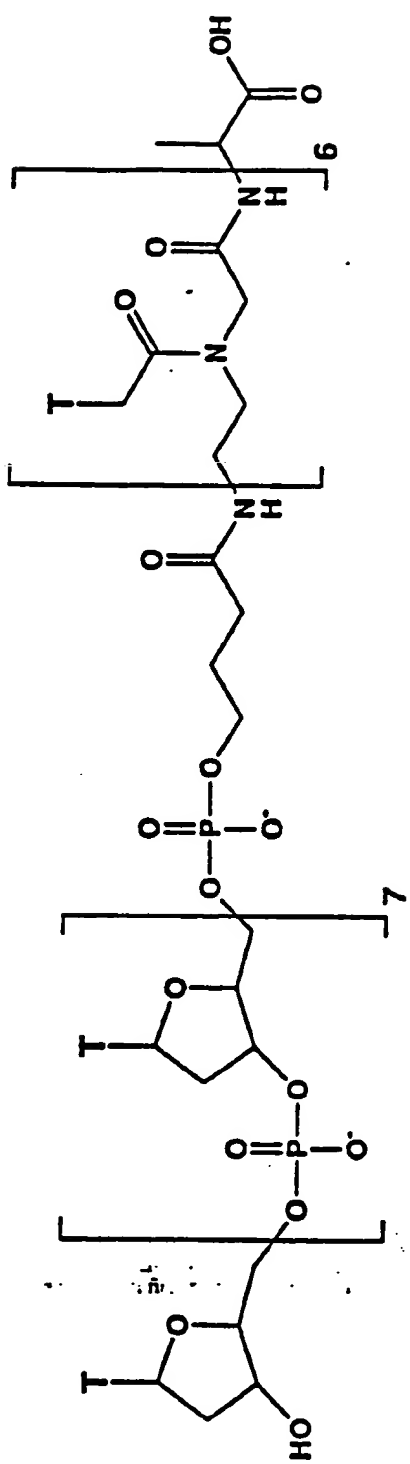


Fig. 25

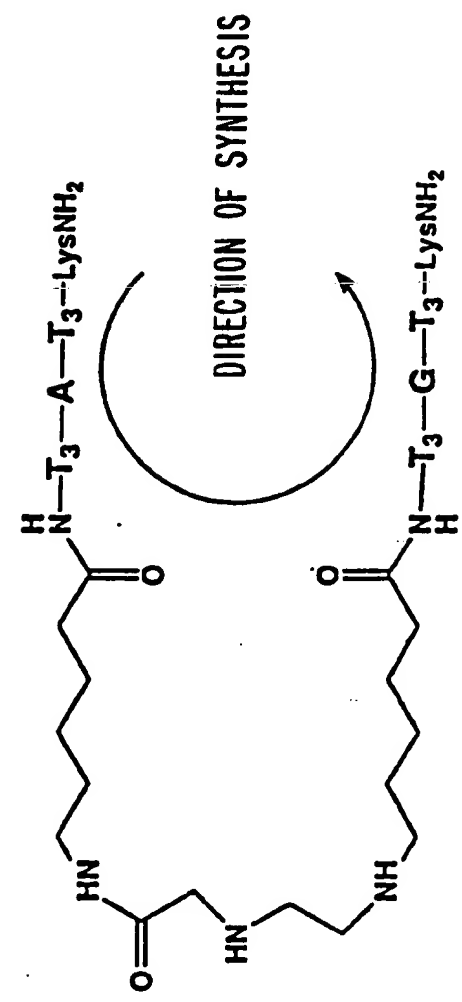
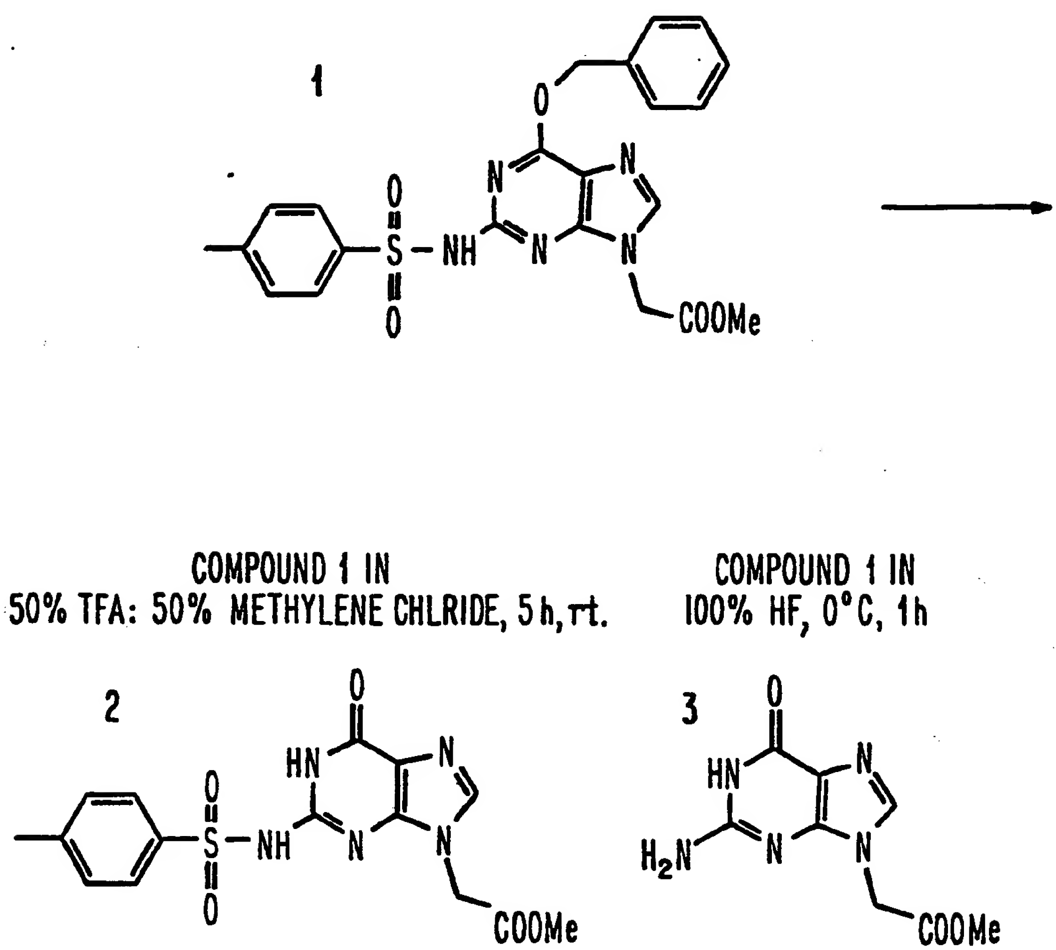


Fig. 26

COMPOUND



QUANTITATIVE DE-BENZYLATION

QUANTITATIVE DE-BENZYLATION AND DE-SULFONYLATION

Fig. 27

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 92/01219

I. CLASSIFICATION F SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 07 K 5/00 C 07 K 7/00 C 12 Q 1/68
C 08 L 77/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 K 5/00 C 07 K 7/00 C 12 Q 1/00 C 08 L 77/00

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
1 X	WO,A,8605518 (J. SUMMERTON) 25 September 1986 (cited in the application) ---	
1 Y	WO,A,8605519 (J. SUMMERTON) 25 September 1986 ---	
5 P,X	Journal of the American Chemical Society, vol. 114, no. 5, 26 February 1992, American Chemical Society, M. EGHOLM et al.: "Peptide nucleic acids (PNA). Oligonucleotide analogues with an achiral peptide backbone", pages 1895-1897 ---	
5 P,X	Science, vol. 254, 6 December 1991, P.E. NIELSEN et al.: "Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide", pages 1497-1500 --- -/-	

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10-09-1992	07. 10. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	

Form PCT/ISA/210 (second sheet) (January 1985)

International Application No PCT/EP 92/01219

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	Journal of The American Chemical Society, vol. 110, no. 22, 26 October 1988, American Chemical Society, D.P. MACK et al.: "Design and chemical synthesis of a sequence-specific DNA-cleaving protein", pages 7572-7574 ---	
A	Journal of Medicinal Chemistry, vol. 33, no. 7, 1990, American Chemical Society, L.P.G. WAKELIN et al.: "Kinetic and equilibrium binding studies of amsacrine-4-carboxamides: a class of asymmetrical DNA-intercalating agents which bind by threading through the DNA helix", pages 2039-2044 ---	
A	BIOCHEMISTRY, vol. 27, no. 17, 23 August 1988, American Chemical Society, P.E. NIELSEN et al.: "Photochemical cleavage of DNA by nitrobenzamides linked to 9-aminoacridine", pages 6338-6343 -----	

International application No.
PCT/EP 92/01219

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)( ) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 23-31 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compounds.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ N protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. EP 9201219
SA 60822

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 24/09/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A- 8605518	25-09-86	AU-A-	5661386	13-10-86
		AU-A-	5698186	13-10-86
		CA-A-	1268404	01-05-90
		EP-A-	0216860	08-04-87
		EP-A-	0215942	01-04-87
		JP-T-	62502338	10-09-87
		JP-T-	62502357	10-09-87
		WO-A-	8605519	25-09-86
		US-A-	5034506	23-07-91
WO-A- 8605519	25-09-86	AU-A-	5661386	13-10-86
		AU-A-	5698186	13-10-86
		CA-A-	1268404	01-05-90
		EP-A-	0216860	08-04-87
		EP-A-	0215942	01-04-87
		JP-T-	62502338	10-09-87
		JP-T-	62502357	10-09-87
		WO-A-	8605518	25-09-86
		US-A-	5034506	23-07-91

09/774203

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82